Universidade de Lisboa

Faculdade de Farmácia

Renal cell carcinoma:

Analysis of the use of oncologic immunotherapy

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Resumo

O cancro do rim é o 9º cancro mais comum no homem e o 14º cancro mais comum nas mulheres. O carcinoma das células renais é a principal forma de cancro do rim e pode ser classificado de acordo com a histologia, morfologia, características de crescimento e mecanismos moleculares respetivamente. Devido à heterogeneidade que apresenta, poucas são as abordagens terapêuticas completamente esclarecidas e com fortes evidências clínicas.

Nivolumab é um anticorpo monoclonal humano de imunoglobulina que se liga aos recetores de morte programada-1 (PD-1) e bloqueia as conexões com os ligantes PD-L1 e PD-L2. O recetor PD-1 é um regulador negativo da atividade das células T que provou estar envolvido no controlo das respostas imunitárias das mesmas. Este fármaco tem demonstrado assim uma promissora atividade anti tumoral em diversos ensaios clínicos.

De acordo com as guidelines da European Society of Clinical Oncology (ESMO) e da National Comprehensive Cancer Network (NCCN), o nivolumab é uma das principais referencias de tratamento em doentes diagnosticados com carcinoma das células renais, nomeadamente em doentes de intermedio ou elevado risco. Adicionalmente, o nivolumab é fortemente recomendado com grandes níveis de evidencia.

Com o objetivo de se estudar os benefícios e os riscos desta inovadora classe terapêutica, foi delineado um trabalho de campo a fim de se estudar as intervenções terapêuticas comparando as mesmas com as guidelines em vigor, bem como compreender como os doentes beneficiam das mesmas. Deste modo, em cooperação com o hospital São Francisco Xavier, uma análise da utilização da imunoterapia oncológica foi realizada em quatro doentes com cancro do rim.

Não obstante o facto de a amostra em estudo ser pequena, o nivolumab provou na maioria dos elementos, benefícios clínicos significantes que demonstraram superioridade em relação ao risco associado à terapêutica com o mesmo. Para além disto, o nivolumab apresentou nesta população heterogénea, consideráveis evidências de eficácia e segurança, assim como, um perfil beneficio/risco positivo.

Por fim, diversos são os desafios por ultrapassar nesta área terapêutica. Muito deverá ser o trabalho futuro a fim de se compreender o papel da imunoterapia oncológica em cada variante do cancro do rim, considerando cada uma como uma doença única e aplicar este conhecimento às diferentes características de cada doente. Igualmente, a identificação de novos e válidos biomarcadores é necessário para suportar as decisões clinicas e prever as melhores respostas.

Palavras-chave: Carcinoma das células renais, imunoterapia oncológica, nivolumab, doente, beneficio, risco.

Abstract

Kidney cancer is the 9th most commonly cancer in men and the 14th most commonly cancer in women. Renal cell carcinoma is the principal form of kidney cancer and can be subdivided into categorizations based on histology, morphology, growth and molecular pathways features. Due to this large heterogeneity, few are the therapeutic approaches completely understood and with strong levels of clinical evidence.

Nivolumab is a human immunoglobulin monoclonal antibody that binds to programmed death receptors-1 (PD-1) and intercept the interactions with PD-L1 and PD-L2 ligands. PD-1 receptor is a negative regulator of T-cell activity that has been proved to be involved in the control of T-cell immune responses. This drug has demonstrated promising antitumoral activity in large clinical trials.

According to the European Society of Clinical Oncology (ESMO) and the National Comprehensive Cancer Network (NCCN) guidelines, nivolumab is one of the main standards of treatment in patients with renal cell carcinoma, in particular in patients with intermediate and poor risk profiles. Additionally, nivolumab is strongly recommend with high levels of evidence.

In order to investigate the benefits and risks of this promising therapeutic class, a fieldwork was delineated to study the therapeutic interventions comparing with the guidelines in force, as well as, to understand how patients benefit from them. In cooperation with São Francisco Xavier Hospital, an analysis of the use of oncologic immunotherapy was performed in four patients with renal cell carcinoma.

Notwithstanding the sample in study was small, nivolumab proved in the majority of the elements, significant clinical benefits that demonstrated superiority in relation to the risk associated. In addition, in this heterogeneous population, nivolumab presented good efficacy and safety evidences, as well as, a positive benefit/risk profile.

There are still several challenges to overcome in this therapeutic area. Further work must be performed to comprehend the role of cancer immunotherapy in each histological subtype of kidney cancer, considering them as single diseases, and apply this knowledge to the different patient profiles. Likewise, the identification of new and valid biomarkers is required to support clinical decisions and predict the best responses.

Keywords: Renal cell carcinoma, oncologic immunotherapy, nivolumab, patient, benefit, risk.

Contents

Figures caption

Tables caption

Abbreviatures

TSAs- Tumour specific antigens

TAAs- Tumour associated antigens

MHC- Major histocompatibility complex

NK- Natural killer

DC- Dendritic cells

VEGF- Vascular endothelial growth factor

PD-1- Programmed death cell receptor

PD-L1- Programmed death cell ligand 1

PD-L2- Programmed death cell ligand 2

HLA- Human leukocyte antigen

APCs- Professional antigen-presenting cell

FDA- Food and drug administration

ESMO- European Society of Clinical Oncology

NCCN- National comprehensive cancer network

RCC- Renal cell carcinoma

ccRCC- Clear cell renal cell carcinoma

non-ccRCC- Non-clear cell renal cell carcinoma

NSAIDs- Nonsteroidal-anti-inflammatory drug

TNM- Tumour, Node, Metastasis

mTOR- Mammalian target of rapamycin

IFN- interferon

IL- Interleukin

1. Introduction

Kidney cancer is among the ten most prevalent cancers in men and among the fifteen most prevalent in women. Although research is growing in this oncology field, there are many challenges to be overcome.

Renal cell carcinoma is the principal form of kidney cancer and can be subdivided into categorizations based on histology, morphology, growth and molecular pathways features. In this way, five main subtypes can be highlighted: clear cell, papillary, chromophobe, sarcomatoid and collecting duct carcinomas, each one with its distinct prevalence in the population.

Due to the wide diversity of the kidney cancer, few are the therapeutic approaches that are completely understood. Furthermore, most of the published studies and available medicines are in the field of clear cell histology, the most common subtype of renal cell carcinomas. So nowadays, one of the biggest problems in this area is that the scientific authors assume the remaining histological subtypes as a homogeneous group and try to apply the results obtained in clear cell patients in this one heterogeneous set.

Moreover, there are slight differences between the main guidelines (European and American) that are consider as a reference in clinical practice. These minor different recommendations must be explored and tested. While on the subject, the majority of the therapeutic options referenced when the standard agents are not available lack of strong evidences of efficacy or significant benefit/risk ratio, as well as, supportive large and conclusive clinical trials. In this way, it is really important that the therapeutic approaches considered as the standard of care, strongly recommended by clear and robust evidences demonstrate noteworthy clinical benefits in clinical practice.

Nivolumab is a human immunoglobulin monoclonal antibody that binds to programmed death receptors-1 (PD-1) and intercept the interactions with PD-L1 and PD-L2 ligands. PD-1 receptor is a negative regulator of T-cell activity that has been demonstrated to be involved in controlling T-cell immune responses. PD-1 receptor binds to PD-L1 and PD-L2 ligands, which are expressed in antigen presenting cells and possible expressed by tumour cells or other cells in the cancer microenvironment, results in inhibition of T cell proliferation and cytokine secretion. Thereby, this immunomodulator target agent potentiates T-cell activity, including antitumor responses by blocking PD-1 connections. Additionally, nivolumab is one of the principal drugs referred as the standard of treatment in these patients and has been proved truly promising antitumoral activity through an innovative mechanism of action.

For these reasons, a work focused on clinical practice was outlined, to understand the methodologies and therapeutic decisions based on the different guidelines and how patients benefit from them. Complementary, the analyse of the results obtained and if they meet the scientific literature are an important topic. Notwithstanding, the main issue is interpreting the outcomes achieved with nivolumab therapy and conclude if the clinical benefit outweighs the risk associated.

This fieldwork will add value to this therapeutic area and contribute to understand the benefits and risks of cancer immunotherapy, a new promising therapeutic approach.

2. Objectives

The purpose of this monograph is to analyse cancer immunotherapy, with the principal focus on the benefit / risk profile of nivolumab, a human immunoglobulin monoclonal antibody.

Additionally, it is also intended to understand how the current guidelines are applied in clinical practice, the outcomes achieved with each therapeutic approach, compare these results with relevant scientific data and strong evidences, and conclude about the role of immunotherapy in patients with renal cell carcinoma.

3. Background

3.1. Why do we have cancer?

Cancer is a cellular disorder consequence of uncontrolled growth of tumour cells. Most of the scientific evidences emphasize that genetic errors, inheritance and environmental factors are the main causes of this disease. In this way, these reports identify random errors in replication of genome as a key role in cancer biogenesis. In children, primary genomic irregularities emerge during embryogenesis before the functional maturation of the immune system, while adult secondary genomic abnormalities emerge in the setting of stress and chronic inflammation conditions. These blunders are responsible of two-thirds of the mutations that originate human cancers and for the fact that these errors are random, most of the malignancies might not be predictable and preventable (1,2).

Human system has internal and external checkpoints to control irregular changes in the development and growth of cells. Once these checking systems fail, tumour cells will proliferate and grow out of control. In early stages, cancer cells display similar appearance to normal cells, however different metabolic and proliferation features, with higher nutrients consume and division rates respectively (1).

The internal checking procedure is mediated by a family of tumour suppressor genes that will activate several enzymes to breakdown the genetic material into short fragments in a process designate “programmed cell death”. The aim of this process is blocking the proliferation and survival of the cells, which mutations are detected and cannot be correct, through a programmed death. Once tumour cells escape to internal check, they will pass to an external one where immune system has the ability to recognize subtle changes in proteins on the surface of these malign cells, with the purpose of destroy them. As long as immune system can ensure these checking procedures, cancer cells cannot evolve into a disease. The opposite is observed when cancer cells escaped to the attack of the immune system and spread throughout the body and eventually end up into death (1).

3.2. Immunogenicity versus immunosuppression

Our immune system subdivides in two main responses: innate and adaptive respectively, which differ in their specificity of recognition and speed of response. Natural killer (NK), macrophages and dendritic cells (DCs) are the major innate immune cells, characterized by a fast response but a poorly specificity. On the other hand, T lymphocytes and B lymphocytes are the central adaptive immune cells, characterized by a higher and restrict specificity in identification of their targets, but with a delayed response (1).

A powerful and competent cancer immunosurveillance requires the expression of tumour specific antigens (TSAs) uniquely present on the surface of tumour cells and, tumour associated antigens (TAAs) express by normal and tumour cells that are able to stimulate T cells proliferation. Thereby, the higher sensitivity of TSAs allows CD8+ T cells to identify tumour specific proteins, whereas TAAs are distinct in each tumour and their recognition requires an

examination of specific mutations and immunogenic epitopes. Recent studies have demonstrate the response of several cancers to immunotherapy approaches, proposing that tumour infiltrating lymphocytes might precisely target TSAs (3).

NK cells were the first innate immune cells identified as the earliest defence in our blood system against metastatic tumour cells. They cytolytic activity is largely regulate by natural cytotoxicity receptors, which in response to upregulate ligands are capable of spontaneously lysing tumour cells without request prior MHC recognition. NK cells have the ability to eradicate tumour cells that have escaped to CD8 + cytotoxic T cells due to the lack of MHC I molecules, a tumour strategy to sidetrack immune system. However, tumour cells slip from NK control trough diverse mechanisms, such as suppression of their activity by downregulate NK-attracting chemokines like CXCL2, reducing the number of NK cells in tumour microenvironment, increase the expression of MHC I to repress NK cytotoxic functions our through the action of a hypoxic milieu as a suppression factor. In addition, three important NK immunosuppression mechanisms are important highlight such as NK and T cells exhaustion by a continuous exposure to tumour antigens, increment of MHC I expression by tumour cells in order to inhibit NK functions, as well as, the regulation of NK by Tregs cells trough the restriction of availability of IL-2. Under physiological conditions, Tregs protect against autoimmune disorders by curb self-reactive cells such as NK cells, T lymphocytes and antigen-presenting cells. In this way, Tregs are activate through the recognition of self-antigens or tumour associated antigens, which leads to the production of interleukin-10 and transforming growth factor (TGF-β), preventing tumour lysis(1,3).

Tumour associated macrophages (TAMs) are the main class of inflammatory cells in tumour milieu, representing up to 50% of the tumour mass and participate in all stages of disease evolution. Tumour hypoxia and chemotactic factors recruit circulating monocytes our tissue local macrophages, that originate TAMs. Generally, macrophages can be subdivide in two groups: M1 macrophages, that produce antitumor response mediators such as TNF-α and IL-12, and M2 macrophages, which produce IL-6, IL-10 and TGF-β involved in tissue healing, remodelling, as well as, in angiogenesis in combination with VEFG secreted by M2 macrophages after M1 turn into a M2 phenotype. This switch is stimulating by factors released from T cells, dendritic cells, Treg cells and tumour cells, which promote tumour burden. This predominately TAMs M2-like profile have demonstrated T-cells suppression abilities and poor antigen presentation competences. Moreover, the migration of macrophages to metastatic locations promote vascular permeability and extravasation of tumour cells through the action of vascular endothelial growth factors (VEGF). In addition, the release of proteases by macrophages destroy the surrounding extracellular matrix and allow cancer cells to migrate (3).

Dendritic cells (DCs) work as a bridge between the innate and adaptive immune responses. These professional antigen presenting cells display antigens to appropriate T cells through a cell surface proteins class named major histocompatibility complex. There are two subsets of MHC: class I and II, which present antigens to CD8 + T cells and CD4 + T cells, respectively (1). Due to this ability, dendritic cells take part of an important role in prompting antitumor responses. In complement to described above, an effective antitumor activity depends of a presentation by mature DCs. The issue is that in tumour milieu, DCs exhibit an immature phenotype, which in turn lead to an insufficient activation of T cells. Furthermore, studies reveal that immature DCs induce suppression cells and produce proangiogenic factors, enhancing tumour growth and dissemination. These properties are repressed by DC maturation (4,5).

Adaptive immunity is divided in two main subsets: cellular and humoral responses, mediate by T cells and B cells, respectively.

Cytotoxic T lymphocytes, the designation of CD8+ T cells, are the primary exterminators of tumour cells through a process mediated by injected enzymes, which cut the genetic material until the apoptosis of these cells. In contrast, the main function of CD4+ T cells, known as T helper cells, is producing cytokine such interleukins. These soluble proteins are responsible for deliver messages between immune cells in order to help or regulate their activity (1).

Before proliferation, T cells demand two signals to be activated: antigen recognition by T-cell receptor (TCR) and co-stimulation link between CD28 and B7 molecules expressed by APCs. As soon as this signalling occurs the survival, metabolization and differentiation rates of T cells increase due to the action of activated interleukins, such as IL-12 (6).

Tregs cells disrupt this CD28-B7 bind by activate a molecule with higher affinity to B7. Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) is an immunoglobulin express on naïve T cells and regulatory T cells, which participate in prevention of autoimmune through downregulation immune response by competing with CD28 to B7, which in turn lead to the block of T cell receptor pathway (1,7). In addition, when T cells are activated express high levels of a member of the B7:CD28 costimulatory family receptors, PD-1. It adjusts T cell activity though the binding of its ligands, programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) in order to prevent impairment to healthy tissues or organs as a result of over-induction of T cells. Nevertheless, this process is the base of tumour adaptive resistance to cancer immunity. Some studies reveal that tumour cells collect a part of interferon gamma as an inducer of their PD-L1 expression (1,8). Thus, immune cells that express PD-1 engage in PD-L1 express in tumour cells. Once dovetailed, the interconnection signals are translated into anergy or death of T cells (1,2).

Distinctly, B cells do not kill malign cells, but produce antibodies, which bind to antigens and neutralize their functions, or boost immune cells like natural killers and macrophages to eliminate target cells that express these antigens. This process is called antibody-dependent-cell-mediated cytotoxicity, a key immune strategy to fight cancer (1).

Besides the suppressive agents and tumour promoting mechanisms detailed above, there are others cells with unique mechanisms of action that must be define, such as myeloid derived suppressor cells (MDSCs), T helper cells (Th17), regulatory B cells (Bregs), cancer associated fibroblasts (CAFs) and tumour associated neutrophils (TANs). Thus, table 1 present a short statement of the main immunosuppressive mechanisms of each cell.

Table 1 – Immunosuppressive cells and the respective mechanism (2).

Cell class	Immunosuppression mechanism
Regulatory T cells (Treg)	Control of autoimmune responses and T cells suppressor effector through negative co-stimulatory pathways.
Myeloid derived suppressor cells (MDSCs)	Immature myeloid cells line that can inhibit NK cells, dendritic cells and effector T cells.
Tumour associated macrophages (TAMs)	Promote the formation of tumour stromal infiltrates.
T helper cell (Th17)	Recruit CAFs, MDSCs and enhance tumour metastasis by cytokines release.
Regulatory B cells (Bregs)	Enhance effector T cells conversion to Tregs and promote tumour metastasis.
Tumour associated neutrophils (TAMs)	Cytotoxic T cells blockage.
Cancer associated fibroblasts (CAFs)	Secretion of suppressor pro-inflammatory cytokines.

3.3. Cancer immunotherapy

Considering the cycle of immune responses against cancer it is possible to identify the main points of failure. In short, immune system can fail in perceive tumour antigens, T cells and dendritic cells track down tumour antigens as self-antigens, suppression of effector T cells production by tumour microenvironment, and the fact that T cells might not be qualified to infiltrate in tumour. The central goal of cancer immunotherapy is restore, preserve and improve this cycle through strategies like improvement of antigen presentation, cells expansion and differentiation and blockage of suppressive processes within tumour sites (9).

Nevertheless, cancer immunotherapy is an exclusive treatment which must be guided by an accurate immunobiographic analysis, where factors such as age, gender, lifestyle and genetics should be taken into account (10). The uniqueness of cancer immune feedback is based on a combination between the specificity and diversity of antigens recognition and presentation, and the variation on type, dose, temporal sequence and intensity of antigens exposure, that is different in each patient (1,10).

In this way, to optimize the therapy for each patient is necessary select the most suitable drug, the ideal dose and identify possible drug resistances and failure mechanisms. In addition, the searching for biomarkers is fundamental to predict and monitor immune responses (1).

3.3.1. Immune checkpoints inhibitors

Human immune system is characterized by its audacity to differentiate self from non-self-cells. This ability is regulated by a counterbalance between co-stimulatory and inhibitory signals, better known as immune checkpoints.(11) These checkpoints pathways control T cell activation and anergy with the aim of prevent autoimmunity disorders through a process designated peripheral tolerance. Thereby, tumour cells develop ways to evade the host by overtake this peripheral tolerance barrier (6,12).

Undoubtedly the research and disclosure of negative regulators of T cell function such as cytotoxic T-lymphocyte-associated antigen (CTLA-4) and programmed death 1 (PD-1) have been essential to the development of cancer immunotherapy (6,12).

Both CTLA-4 and PD-1 connections have similar negative consequences on T cell function. However, the time of action, the anatomic locations of immune suppression and the signalling procedures are distinct. In addition, the distribution of these immune checkpoints ligands also differs. CTLA-4 ligands are express by professional APCs, which commonly reside in lymph nodes or spleen, while PD-L1 and PD-L2 are more widely disseminated. PD-L1 is found on leukocytes and nonlymphoid tissues and can be activated by tumour signalling molecules or inflammatory cytokines, whereas PD-L2 also is induced by a large range of immune and nonimmune cells, but expressed on monocytes and dendritic cells (6).

Additionally, CTLA-4 functions as a primary immune response, at T cells dependent areas such as lymph nodes, in order to regulate the activation and proliferation of T cells, as well as, to control potential autoreactive disorders. On the other hand, PD-1 pathway works to restore the immunity in later stages at effector sites, like chronically inflamed tissues or advanced tumours, supressing T cells already activated (6,11,12).

Preliminary trials have demonstrate that immune checkpoints inhibitors boost the survival of patients with advanced malignancies, such as melanoma, urothelial bladder cancer, non-small cell lung cancer and renal cell carcinoma (11). For this reason, several new drugs have been approved, such as ipilimumab (anti-CTLA-4), pembrolizumab (anti-PD-1) and nivolumab (anti-PD-1). Both are fully human monoclonal antibodies that can improve antitumor responses (6,11). Pembrolizumab has been used in the treatment of advanced melanoma, non-small cell lung cancer and advanced urothelial bladder cancer, while nivolumab has demonstrated efficacy on advanced melanoma, non-small cell lung cancer and renal cell carcinoma (11). Furthermore, several studies suggest that simultaneous inhibition of CTLA-4 and PD-1 display superiority when in comparison with CTLA-4 or PD-1 blockade alone or in sequence (6).

Nevertheless, despite these medicines can improve progression free survival and overall survival in a great part of the patients with advanced tumours, a significant proportion do not benefit from immunotherapy. Unfortunately, some tumours are so powerful that the suppression and resistance to therapeutic overrule. Thus, it is imperative more research and improving of knowledge in this field (13).

Also, in clinical practice some immune related adverse events to immune checkpoints inhibitors are reported. Usually, the spectrum of toxicities embrace rash, gastrointestinal disorders, pneumonitis, hepatitis, nephritis, colitis, haematologic syndromes and

endocrinopathies (11,14,15). Generally, these adverse effects occur within two weeks to three months after immune checkpoint inhibitor first`s dose (11).

Currently, identifying biomarkers is crucial to clinical decisions and select the most suitable treatment for a specific patient. Low levels of CTLA-4 and broad expression of its B7 ligands are not effective predictive biomarkers. In counterpart, the high levels of PD-1 expression on exhausted cells and PD-L1 on tumour cells or tumour-infiltrating immune cells might be potential biomarkers to select patients responsive to PD-1 blockade (6,16). Preliminary reports suggest that expression of PD-1 and its ligand PD-L1 as prognostic biomarkers predict poor outcomes in these patients (16). On the other hand, PD-L1 expression varies from the primary to metastatic areas and its heterogeneity is promoted by tumour environment factors, which can lead to controversial uses in clinical practice (1,17).

To clarify the concepts introduced earlier, figures 1 and 2 are presented.

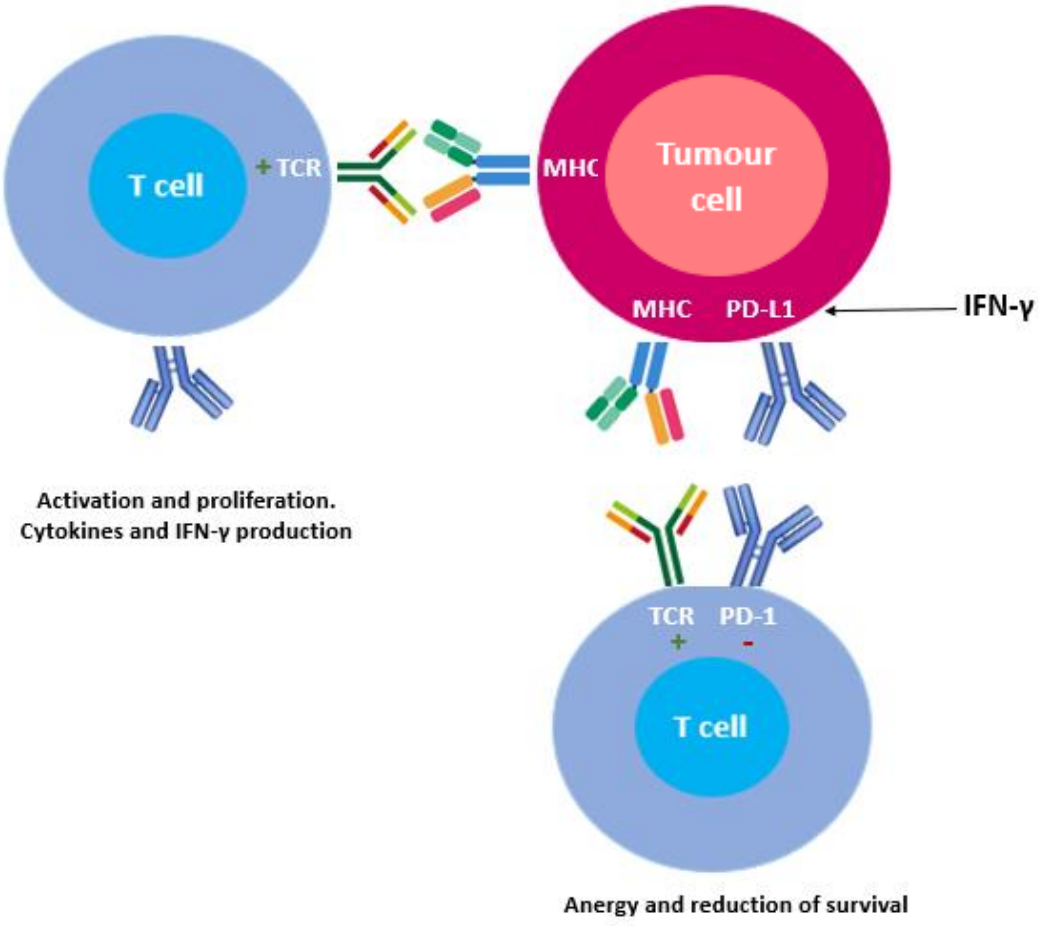

Figure 1 – PD-1/PD-L1 immunosuppression mechanism. MHC displayed antigens, which are recognized by T cell. This signal potentiates T cell proliferation and activity. Some tumours can use part of the IFN-γ circulating to induce the PD-1/PD-L1 pathway. Anergy and poor survival are the main consequences in T cells. Adapted from (6).

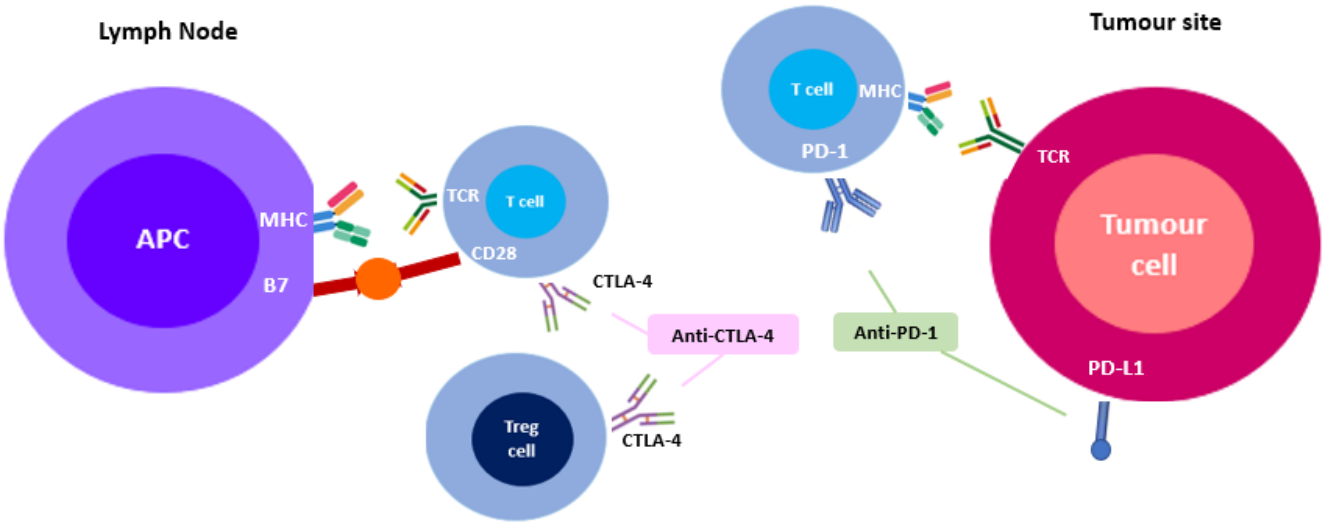

Figure 2 – Immune checkpoint inhibitors mechanisms. CTLA-4 blockade promotes activation and proliferation of T cells, as well as, neutralize the suppression activity of Treg cells. Complementary, PD-1 blockade restores the function of effectors T cells. The synergistic effects of these two inhibitor drugs lead to a higher tumour elimination. Adapted from (6).

3.3.2. Tumour microenvironment target therapy - Vaccines

Vaccines are prophylactic agents, which provide long term immunity against one or several pathogens, preventing diseases and outbreaks.

Nowadays, in the field of immunotherapy against cancer, prophylactic vaccination plays an important role in virally induced malignancies, such as human papilloma virus (HPV) and hepatitis B virus in genital and liver tumours respectively (18). In contrast to a neutralization process mediate by antibodies, cancer vaccines must act by potentiate tumour specific immune responses to avoid the incidence and development of spontaneous and non-microbially tumours. Cancer vaccines must promote an effective activation of T cells and built long term anti-tumour responses based on tumour specific memory cells (19).

The main drawbacks are the lack of an ideal antigen and the efficacy of these vaccines. The real problem is that immune system is already tolerized to tumour antigens, which hamper the antigens-vaccine design. In recent years, distinct non-tolerogenic tumour associated antigens have been discovery and some of them include antigens derived from oncogenes mutations (20). In addition, antagonistically to viral ones, tumour antigens have a large variation range according to cancer type and the different patients (21). Therefore, an ideal tumour antigen must be indispensable to tumour growth or survival, expressed in a broad number of patients and not find in healthy tissues (22).

Unsuccessfully, the first approaches have failed to demonstrate clinical benefits. However, the efficacy of these vaccines has improved over the years, and in 2010, FDA authorized the first

vaccine for cancer treatment. The Sipuleucel-T was approved for patients with metastatic castration-resistant prostate cancer (20).

The first strategy to develop therapeutic vaccines was based on "whole tumour cells" and the subsequent combination with immunostimulatory cytokines was a remarkable advance. The primary limitation of these method is the acquisition of patient specific cells in a massive amount. Bypassing this limitation and continue target the maximum TAAs, peptide vaccines were developed. This approach can simply and economically produce and administered into patients with non-toxic relative effects when compared to whole-cell vaccines. Nevertheless, the higher disadvantage is that these peptides must be compatible with HLA molecules, derived from TAAs expression, to generate cytotoxic responses. Considering that cancer cells can change their immunogenic antigens, unsatisfactory outcomes led researchers to develop dendritic cell based vaccines (20). Dendritic cells are the most specialized APCs for promote tumour specific and effective T cell responses. In this way, distinct strategies were tested, such as in vivo DC-targeting (anti-DC fused to antigen) and dendritic cell vaccination (adoptive transfer of DCs isolated from patient`s blood, stimulate with tumour antigens and reinjected into the patient), sometimes associated with adjuvants aimed to deliver activation signals to the immune system. These therapeutic vaccines have demonstrated better clinical results (19,20).

Despite T cells specific activation and proliferation, therapeutic vaccines have remained ineffective due to tumour induced resistances. Findings of preclinical and clinical studies have demonstrated that the combination of therapeutic vaccines with other treatment approaches like radiotherapy, hormone therapy, chemotherapy and immunotherapy produce successfully clinical outcomes (18).

3.3.3. Tumour microenvironment target therapy - Oncolytic virus therapy

Oncolytic virus are a new class of cancer biotherapeutics agents that foster infection and elimination of tumour cells without impair healthy tissues, through a dual method of action: selective tumour cells death and enhancing antitumor immune responses. By contrast to classical viral based vaccines, oncolytic viruses directly lyse and kill tumour cells in situ. Herpes simplex virus, coxsackieviruses, poliovirus, adenovirus, measles virus and Newcastle virus are some virus strains designed and manipulated for selectively replicate within neoplastic cells without significant toxicity for healthy cells. The lytic ability of oncolytic viruses is reliant on the virus strain and tropism, dose, the type of tumour cells and their susceptibility, as well as the tumour-host interaction and activation of innate and adaptive antitumor immune responses (23).

Recent evidences reported that the use of oncolytic virus's immunotherapy following the immune checkpoints inhibitors treatment demonstrated favourable results. The death of neoplastic cells promotes a pro-inflammatory environment and the release of hidden neo-antigens. Hence, these antigens are identified by APCs and new T cells clones are generate and able to kill tumour cells that were not identified before and the ones, whose were not infected by the virus. Nevertheless, several disadvantages are possible to highlight, such as pre-existing neutralizing antibodies and specific memory T cells boost by the host antiviral immune response, which promote viral clearance, and likewise, tumour size, heterogeneity and type of

microenvironment that condition the oncolytic activity. In addition, physical obstacles that limit the biodistribution of oncolytic viruses comprise hypoxia, necrosis, acidosis, high interstitial pressure and a large extracellular matrix (poorly vascularized). The most part of clinical data have used intratumoral injections (which are limited to tumour affordability) to overtake these tumour barriers (23–25). Furthermore, these clinical studies reveal that the kinetics of activation, proliferation and lysis of neoplastic cells might be slower in relation to therapeutic approaches that directly kill tumour. For these reasons, a considering promising combination is oncolytic viruses with checkpoints inhibitors, which extend the efficacy and range of cancers targets (23).

3.3.4. Tumour microenvironment target therapy - Cytokines

Cytokines are molecular signalling mediators of the immune system that generate and regulate a powerful, multifaceted and efficient response to a specific antigen. Aside from important functions such as cellular expansion, proliferation and survival, cytokines are responsible for immune homeostasis, and for these reasons are crucial for maintain tumour surveillance. Despite boost tumour cells recognition and promote cytotoxic effectors response, these molecular messengers display a noticeable function in the balance between tumour escape and its elimination (26,27). Thus, cytokines are classify into two main subsets if they are correlate with acute inflammation and antitumor activity (type 1) or with suppressive tumour effects (type 2) (2).

To date, two cytokines have been approved by FDA for cancer treatment: high dose of IL-2 for metastatic melanoma and renal cell carcinoma, and IFN-α as adjuvant therapy for stage III melanoma (28). In these two types of cancer, IL-2 demonstrated durable responses and in particularly, in clear-cell renal carcinoma that resist to cytotoxic drugs, the therapy with IL-2 and IFN-α have achieved good responses and durable remissions in several patients with distinct risk stratification classifications (26,29). Nonetheless, cytokines are able to act on distinct cells, mediate several and occasionally, opposite effects. This competence is known as pleiotropism and is the principal limitation of IL-2 therapy, since this cytokine is capable dually stimulate a potent T effector immune response, as well as, a T-cells regulatory activity (26).

Members of an important cytokine's family based on commonly receptors, known as γc receptors subunits have been broadly studied and demonstrated good responses in selected malignancies. In this way, IL-2, IL-4, IL-7, IL-9, IL-15, IL-21 and thymic stromal lymphopoietin (TSLP) can generate overlapping but unique signals, which culminate in the activation and proliferation of CD4+ and CD8+ T cells (26,27).

Thereby, in the table below mentioned (table 2), it is possible to verify that each cytokine triggers a single cellular response in common targets that will promote a synergistic antitumor response.

Table 2 – Type of cytokine, cell source, target cells and cellular function respectively.

Cytokine	Cell source	Target cells	Cellular function
IL-2	Th, DC and NK cells.	T, B, NK cells and monocytes.	Dual function as an immunomodulator (30). Promotes differentiation and proliferation of effector T cells, B cells and NK cells (27). Elimination of autoreactive T cells by Treg cells based immunosuppressive strategies (31).
IL-4	T and NK cells; mast cells and eosinophils.	T, NK and B cells; mast cells and basophils.	Differentiation of naïve CD4+ T cells into Th2 cells (27,32). Stimulation of B cells and immunoglobulin class switching (27,32). Chemotaxis of mast cells, eosinophils and basophils (33).
IL-7	Epithelial and stromal cells; fibroblasts.	T, B and DC cells.	Survival and growth factor of naïve and memory T cells (34). Enhance T cell activity, diversity and homeostasis (26,27,34).
IL-9	T cells.	T cells; epithelial and mast cells; eosinophils.	Activation and proliferation of CD4+ T cells, B cells, eosinophils and mast cells (27).
IL-15	Neutrophils, monocytes, DCs, B cells, mast cells, and fibroblasts.	T, B and NK cells; fibroblasts.	Promote activation, development, maturation and survival of T, B and NK cells by upregulation of anti-apoptotic pathways and downregulation of pro-apoptotic agents (35). Improve DC presentation and induction of T cytotoxic cells (36). Generation, maintenance and reactivation of naïve, effector and memory T cells (35). Activity regulation of neutrophils and macrophages (35).

IL-21	T cells and NK cells.	T, B and NK cells; DCs.	Promote T and NK cells activation and differentiation, as well as CD8+ T and NK cells cytotoxic responses (37,38). B cells proliferation and differentiation into plasma cells (39).
TSLP	Keratinocytes, fibroblasts, stromal cells, mast cells, basophils.	T, B, NK cells and DCs; mast cells.	Activation of CD4+ and CD8+ T cells antitumor immunity, as well as B cells and mast cells expansion (40,41).

Nonetheless, cytokines-based therapy has a significant toxicity associate, so is necessary to select the patients that will more benefit and direct research for predictive biomarkers in this field. Furthermore, combinations regimes that target distinct and multiple pathways while minimize adverse effects have demonstrate good antitumor responses in patients with renal cancer (26,42). Vaccination and adoptive cell therapies are an example where the use of cytokines is crucial for *ex vivo* cell generation, whereas in combination with checkpoints inhibitors, cytokines play a key role in *in vivo* augmentation of effective and durable antitumor responses (26).

3.3.5. Adoptive T cells

Adoptive T cells therapy (ACT) is a personalized cancer immunotherapy that requires the selection and isolation of tumour reactive lymphocytes, their *ex vivo* expansion and subsequently reinfusion into patient. These natural host cells genetic modified are able to proliferate in vivo and generate durable antitumor responses (43,44).

Heretofore, there are three main therapies advancing on approval: tumour-infiltrating lymphocytes (TILs), chimeric antigen receptor T cells (CAR-T) and T-cell-receptor engineered T cells (TCR) (45). Focusing attention in each approach, host lymphocytes infiltrate tumour are usually incapable to reduce cancer burden due to suppression milieu present, and TCR engineered cells are dependent of MHC antigen recognition, which in turn is downregulate by tumour as an escape strategy (44,46). Since CAR-T cells engineered for specific antigens can be use in patients regardless of their HLA type and MHC antigen recognition, is considered the most promising approach. Nevertheless, CAR-T cells present some drawbacks since TCR and MHC signalling pathways can recognize intracellular proteins, whereas CAR-T are only enabled to identify surface tumour proteins (44). In addition, the challenge in this moment is select the best non-mutated antigens that are overexpress on neoplastic cells but not on normal tissues to produce on-target effects with minimal toxicity (43).

Promising outcomes with CAR-T cells therapy in blood cancers, such as B-cell leukemias and lymphomas have raised the widespread use of CAR-T cell in clinic. However, in the treatment of solid tumours the challenge is more complex and the results are lower than expected (47).

Apart from distinct solid cancers have different tumour associated antigens and the inability to traffic into the tumour and overtake the immunosuppressive microenvironment, the target cells are not specific B cells as in blood cancers. In this class of neoplasias, CD19 CAR-T cells is the main technique apply in B cell malignancies clinical trials, by reason of the fact that CD19 is a specific marker identified in all stages of B cells differentiation and not a tumour associated antigen, which is translated into a superior ability to connection. Besides that, the negative tumour microenvironment in hematologic cancers is inferior, leading to excellent benefits to these patients (47,48).

Due to the issues mentioned above and the fact that the majority of cancers develop a series of strategies to escape to immune killing effectors, the future in solid malignancies is combining T cells checkpoints inhibitors with adoptive T cells in order to obtain synergistic effects (44,45).

3.4. Renal cancer

Kidney cancer is the 9th most commonly cancer in men and the 14th most commonly cancer in women (49). In 2018, in Europe was estimated 3.91 million new cases of cancer and 1.93 million deaths, whereas in 2019, in United States was estimated 44.120 new cases of renal cancer in men and 29.700 in women, respectively (49,50).

Globally, the incidence of renal tumours differs widely from region to region, with the highest rates detected in developed countries (51). However the incidence rates are still increasing, most countries have achieved stable mortality trends (52). In addition, renal tumours are approximately 50 percent more common in men compared with women and it is rare in patients younger than 40 years of age and in children (53–55). The majority of kidney tumours among children is nephroblastoma (Wills tumour), enclose 1.1% of all kidney cancers (51).

Primary renal neoplasias, developed within renal cortex, constitute 85% of parenchymal renal cell carcinomas. The remaining 15% are classify as transitional cell carcinomas, divided into renal pelvis and renal capsule tumours respectively (56). Furthermore, renal cell carcinomas classification is based on histology (figure 3), morphology, cell of origin, molecular structure and growth pattern, which provide a RCC categorization into different subtypes, comprising: clear cell (75 to 85 percent of tumours), papillary (10 to 15 percent), chromophobe (5 to 10 percent), sarcomatoid (3 to 7 percent) and collecting duct/medullary (very rare) cancers (57,58).

Clear cell carcinomas, the most common subtype of RCC, typically arise from the proximal tubule and are characterized by a deletion of chromosome 3p and/or a mutation in the von Hippel-Lindau (VHL) gene at chromosome 3p25 (59). In the same way, papillary RCC, the second most commonly subtype identified in clinical practice, has been subdivided into two subsets with distinct clinically and biologically features: type 1 traditionally correlate with activation of MET mutations and generally present in stage I or II of the disease, where patients have relatively better prognosis, and type 2 usually associate with NRF2-antioxidant response element (ARE) pathway activation and mutations in fumarate hydratase (FH) genes, which stage III or IV are the predominant presentations and patients have worse prognosis (60). On the other hand, chromophobe carcinomas, oncocytomas and collecting duct tumours appear to originate from the intercalated cells of the collecting ducts, which the collecting duct cancer subtype is the prevalent in younger patients and represent the most aggressive variant (61–63).

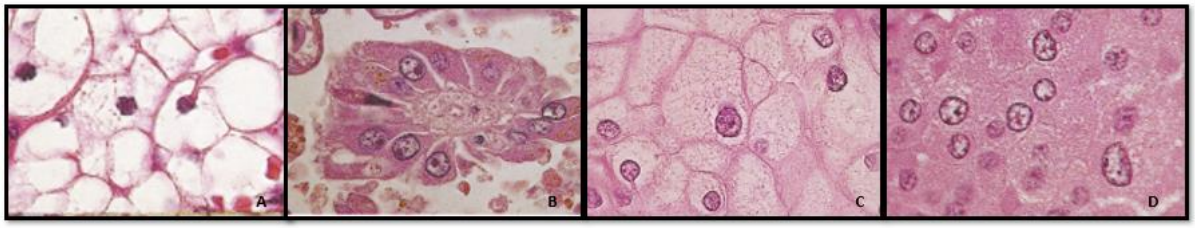

Figure 3 – Histology of the distinct subtypes of renal cell carcinoma. A) Clear cells; B) Papillary cells; C) Chromophobe cells; D) Oncocytes. Adapted from (58).

3.4.1. Risk factors

Few risk factors are stablished for renal malignancies, however some of them are strongly correlate with RCC such as smoking, overweight, hypertension and germline mutations (51,64,65).

Cigarette smoking is recognized as a causal risk factor for renal cell cancer by the International Agency for Research on Cancer (IARC) (51). Data have been shown that massive smoking habits increase the likelihood of advance diseases, whereas durable cessation mitigate this risk (66). Complementarily, smoking habits were associate to upsurge of renal cancer risk, through a chronic hypoxia process mediate by carbon monoxide exposure, and smoking-related pathologies like chronic obstructive pulmonary disease. In this way, some studies reveal that renal cell patients have a higher stage of DNA damage in their peripheral blood lymphocytes increase by a specific N-nitrosamine, as well as, several deletions in chromosome 3p (a standard site of genetic mutations in renal cell cancer) when in contact with major constituents of tobacco (51). In addition, RCC related mortality indicate that this risk factor might not only be correlate with renal carcinogenesis, but also with cancer progression (66).

Likewise, high body-mass index and hypertension are considered independent risk factors of renal tumours in a dose-response manner in both men and women (67–69). Thus, excess body weight has been estimated to contribute for over 30% of kidney cancers in Europe and over 40% in the United States, whereas history of hypertension has been associated with 67% increased risk, respectively (51,70). Next in order, data have been linked diabetes mellitus to renal cell cancer, however it remains unclear if diabetes is an independent factor or an intermediate step between predisposing conditions (hypertension and obesity) and renal pathologies (51,71).

In addition to clinical features, patients with end-stage renal disease undergoing long-term hemodialysis or renal transplantation have higher chance to develop renal cell cancer (51). As well, in several pertinent data, patients with acquired cystic kidney disease demonstrated to have up to 50-fold increased risk to progress to renal cell carcinoma compared to the general population (72). Furthermore, extensively studies have demonstrated that longer durations of non-aspirin NSAIDs therapy may rise the risk of RCC, whereas aspirin and compounds containing phenacetin (of which acetaminophen is a major metabolite) were not correlate with RCC development (73).

Generally, renal cell cancer is not classified as an occupational disease, howbeit some industrial agents have been associated with higher cancer risk. Trichloroethylene, the most chemical compound broadly studied in renal malignancies is categorized by IARC as a probable human carcinogen in group 2A (51,64). Another important factor is the previously exposure to radiotherapy and cytotoxic chemotherapy, which predispose to renal translocation carcinomas (74,75). Studies suggest that children who survive to cancer have a higher risk of developing another malignancy (75).

Finally, although most RCCs are sporadic, few of them are inherited and notably associate with the von Hippel-Lindau syndrome, a rare condition characterized by mutations in the VHL gene, localized into chromosome 3p. Moreover, this syndrome plays an important role in the development of clear cell RCC. Likewise, the genetic factors contribute to an early onset renal cancer so is fundamental identify the hereditary contribution in patients family (51).

3.4.2. Diagnosis evaluation

In the last years, incidental diagnosis of RCC has risen significantly because of radiologic procedures performed for other clinical indications. Thus, the increased of casual detection has improved the survival of these patients due to earlier detection of stage I disease (76,77).

The principal techniques use for diagnosis evaluation are computed tomography (more sensitive), ultrasonography and magnetic resonance imaging (76).

3.4.3. Clinical assessment

The classical triad of clinical manifestations of RCC is flank pain, a palpable abdominal renal mass and hematuria, and typically is related with advanced disease (56).

Generally, the additional RCC presenting symptoms and signs are non-urologic systemic effects such as fever, hypertension, paraneoplastic symptoms, anemia, hepatic dysfunction, polycythemia, hypercalcemia and secondary amyloidosis (78). It is important to highlight the differences between these clinical manifestations, since symptoms like fever, weight loss, anemia and hepatic dysfunction are also seen in other pathology conditions (78). These symptoms may be a result of tumour activity (78). Another preliminary point is that renal tumours can naturally produce erythropoietin, which contribute to polycythemia presentations, and are able to enhance ectopic production of several hormones like parathyroid hormone-related protein, gonadotropins, renin and adrenal cortical hormones, which promote symptoms of Cushing syndromes, hypertension and the increase of calcium in serum (78–81). Likewise, pertinent data outline the role of IL-6 as an enhancer bone resorbing factor, which contribute equally to hypercalcemia conditions (82).

In the most part of the cases, the removal of tumour can attenuate or even eliminate these effects (55,78).

3.5. Staging and classification systems

Tumour, Node, Metastasis (TNM) staging classification system is extensively recommended in clinical practice for prognostic evaluation and choice of patient`s therapy. This system is support by International Union for Cancer Control (UICC) and American Joint Committee on cancer (AJCC). TNM classification structure is based on anatomical extent of tumours (T category), regional lymph nodes involvement (N category) and the presence of distant metastases (M category) (83). Along these lines, the dimension of tumours is divided in T1 and T2 if tumour is limited to the kidney, T3 if major veins or perinephric tissues are comprised but not the ipsilateral adrenal gland and Gerota fascia, and T4 if tumour extend beyond Gerota fascia. Additionally, nodal and distant metastases are generally categorized as absent or present (84).

In the following table is possible analyse the different classifications categories and understand the TMN stage groups respectively.

Table 3 – 2017 Kidney cancer TNM staging classification system (84).

T - Primary Tumour			
TX	Primary tumour cannot be assessed		
T0	No evidence of primary tumour		
T1	Tumour ≤ 7 cm or less in greatest dimension, limited to the kidney		
	T1a	Tumour ≤ 4 cm or less	
	T1b	Tumour > 4 cm but ≤ 7 cm	
T2	Tumour > 7 cm in greatest dimension, limited to the kidney		
	T2a	Tumour > 7 cm but ≤ 10 cm	
	T2b	Tumours > 10 cm, limited to the kidney	
T3	Tumour extends into major veins or perinephric tissues but not into the ipsilateral adrenal gland and not beyond Gerota fascia		
	T3a	Tumour grossly extends into the renal vein or its segmental (muscle-containing) branches, or tumour invades perirenal and/or renal sinus fat (peripelvic fat), but not beyond Gerota fascia	
	T3b	Tumour grossly extends into the vena cava below diaphragm	
	T3c	Tumour grossly extends into vena cava above the diaphragm or invades the wall of the vena cava	
T4	Tumour invades beyond Gerota fascia (including contiguous extension into the ipsilateral adrenal gland)		
N - Regional Lymph Nodes			
NX	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastasis		
N1	Metastasis in regional lymph node(s)		
M - Distant Metastasis			
M0	No distant metastasis		
M1	Distant metastasis		
TNM stage grouping			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1	M0
Stage IV	T4	Any N	M0
	Any T	Any N	M1

In addition, it is also valuable for accurate patient counselling and ponder the risk of treatments another prognostic model based on risk factors. Thus, the International Metastatic RCC Database Consortium incorporated six independent predictors of poor survival in this model: Karnofsky performance status of less than 80%, less than 1 year from diagnosis to treatment, haemoglobin concentration lower than the limit of normal (anaemia) and serum calcium values (hypercalcemia), neutrophil count (neutrophilia) and platelet count (thrombocytosis) upper than the limit of normal respectively. In agreement with the number of prognostic factors, patients are classified into favourable (no factors), intermediate (one or two factors) and poor (more than two factors) risk groups (85).

In this way, the introduction and/or the choice of treatment must take into consideration the tumour localization and dimension, as well as, the patient`s prognostic features.

3.6. State of the art of immunotherapy in renal cell carcinoma

Renal cancer has long been recognized as a chemoresistant tumour but highly sensitive to immunological approaches. Thus, the first efficacy results were reported with IFN-α in 1989, however with limited durable responses and significant side effects. Consecutively, IL-2 was approved in 1992 based on durable and significant responses. Nevertheless, this potent stimulator of T cell activity comprised several adverse effects in multiple organ systems and the arduous affordability for administration in hospital setting (86).

In this way, since 2005 several specific target drugs have been approved for metastatic RCC (figure 4), in particular six medicines that target the tyrosine kinase of vascular endothelial growth factor (VEGF) receptors (sunitinib, sorafenib, pazopanib, axitinib, carbozantinib and levantinib), two that neutralize mammalian target of rapamycin (mTOR) receptors (temsirolimus and everolimus) and one VEGF monoclonal antibody (bevacizumab) (1,87).

In the last years, antibody-based immunotherapies against CTLA-4 and PD-1 immune checkpoints receptors have proved noteworthy efficacy in metastatic RCC, leading to FDA approval of Ipilimumab in 2011 and Nivolumab in 2015 (86,88). Recently, in 2018, the combination of Nivolumab and Ipilimumab was approved by U.S. Food and Drug Administration for treatment of intermediate and poor risk patients (89).

Furthermore, promising clinical trials are ongoing with the aim of study another suitable therapies in this field of immunotherapy such as vaccines, oncolytic virus and adoptive cell therapy. Although the good survival outcomes that have been published, none of these therapies were already approved due to unclear clinical efficacy profile (86,90,91).

The treatment of RCC have been quickly evolved, focus now in the combination of immunotherapeutic agents capable of generate excellent overall survival results and enduring responses. The challenge in this moment is identify reliable biomarkers that predict the best treatment benefits for patients and guide clinical decisions (86,92).

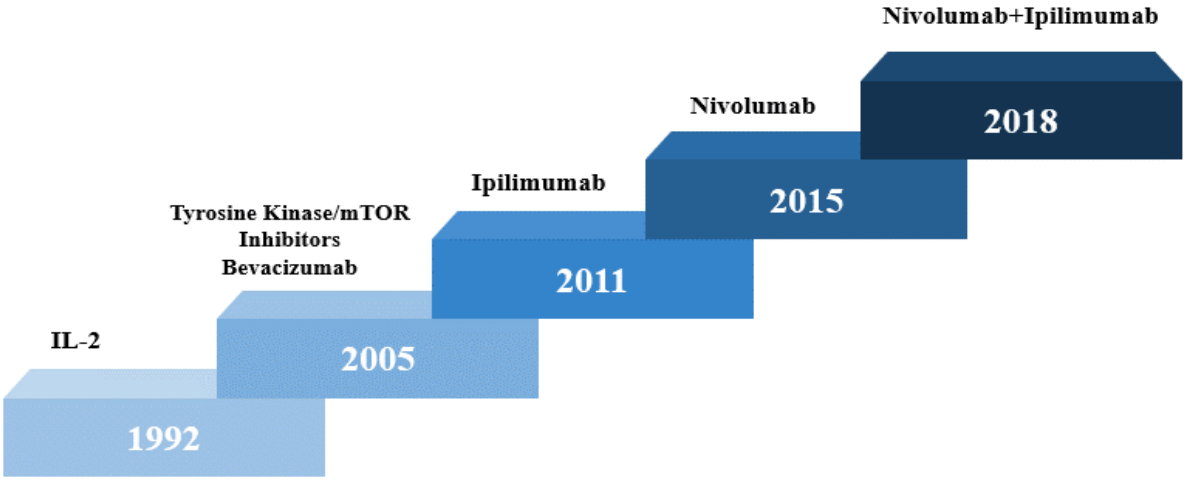

Figure 4 – State of the art of immunotherapy in renal cell carcinoma. The figure displays the respective approval year of each therapeutic approach.

3.7. Role of local therapy in locoregional renal cell carcinoma

The most part of kidney cancer are early identified incidentally by imaging procedures and the majority are small renal masses measuring less than 4 cm. Even though that metastases can be present, generally these localized small masses grow slowly and rarely progress. For these reasons, active surveillance is an option in patients with meaningful comorbidities and/or short life expectancy in attempt to avoid the morbidity of ablative or surgical procedures. Likewise, patients with limited tumour burden and insufficient symptoms should be considered a period of observation before the introduction of therapy (93).

According to the European Society of Medical Oncology, tumours confined to the organ and measuring up to 7 cm is recommend partial nephrectomy. On the other hand, tumours measuring more than 7 cm, laparoscopic is the preferred treatment. Moreover, patients with evidence of adrenal or lymph node invasion, approaches such as systematic adrenalectomy or lymph node dissection can be considered (94).

3.8. Systemic therapy for clear cell RCC histology

Since clear cell histology is the predominant among population, the majority of pivotal trials were conducted in this subtype of kidney cancer. Additionally, treatment recommendations will diverge based on histology and patient`s risk stratification (94).

First-line treatment of clear cell tumours is divided into subsets. If patients were classified into good risk, sunitinib, pazopanib, bevacizumab combined with interferon and tivozanib are the standard of treatment, and high-dose of interleukin-2 and low-dose of interferon combined with bevacizumab are present as treatments options when the first ones are not available. On the other hand, patients classified into intermediate risk, nivolumab combined with ipilimumab is

the standard of treatment and options such as cabozantinib, sunitinib, pazopanib, tivozanib and bevacizumab combined with interferon are indicated. As well, in poor risk patients, nivolumab combined with ipilimumab is the standard of treatment and cabozantinib, sunitinib, pazopanib and temsirolimus are recommend as alternatives (94).

Clearly, vascular endothelial growth factor receptor-tyrosine kinase inhibitors monotherapy are the front-line therapy in patients with good risk features (92). Indeed, interleukin-2 and interferon alfa were largely used in the past as first-line treatments of metastatic disease. However the response rates observed are small and the median overall survival is approximately 12 months, while sunitinib studies reported higher response rates and about 18 months of overall survival (95,96). Furthermore, a large multicentre phase III trial studied previously untreated patients with metastatic clear cell histology and with distinct risk stratification profiles, where randomized 750 patients received a 50 mg daily dose of sunitinib for 4 weeks, followed by 2 weeks without treatment (6-week cycles: 4-weeks-on-2-weeks-off schedule) or subcutaneously interferon alfa given three times weekly. This clinical trial report a higher progression free survival in the sunitinib arm (11 months) than in interferon alfa arm (5 months), as well as, differences in objective response rates that favoured sunitinib. Also, improvements in overall survival demonstrate that sunitinib (26.4 months) represent advantage over interferon alfa therapy (21.8 months) (95,97). In addition, adverse effects such as leukopenia, neutropenia, thrombocytopenia, hand-foot syndrome, diarrhea and hair discoloration were report in the sunitinib group, whereas for the interferon alfa group symptoms like fatigue, pyrexia and myalgias were the prevalent (98)(99). Moreover, phase II data suggest that intermittent treatments with sunitinib are possible and have clinical efficacy in patients with metastatic RCC (100).

Pazopanib, another oral VEGF receptor and platelet-derived growth factor receptor inhibitor was subsequently assessed in a randomized phase III trial in comparison with placebo in 435 treatment-naïve or cytokine pre-treated patients (101). At a dose of 800 mg daily, pazopanib demonstrated longer progression free survival than placebo (9.2 months versus 4.2 months) and noteworthy improvements in tumour response rates (92,101). Further, adverse effects such as hypertension, nauseas, diarrhea, hair colour alterations, anorexia, and the most common, alanine (ALT) and aspartate transaminase (AST) levels elevation, were report in pazopanib group (101). Interestingly, clinical studies have documented that Pazopanib is an active agent after failure of other targeted therapies and/or progression of clear cell carcinoma, and for these reason should be considered in an early treatment line (102)(103).

Along these lines, efficacy and safety of sunitinib and pazopanib in first-line setting were compared in a non-inferiority phase III trial, which 1110 patients with clear cell carcinoma were randomized. In the primary endpoint pazopanib achieve a progression free survival of 8.5 months versus 9.5 months of sunitinib, demonstrating noninferiority. In addition, no differences in overall survival were related. It was possible to conclude that sunitinib and pazopanib have identical efficacy, although the safety and quality of life data support pazopanib, which the most common adverse effects were the increased of transaminases, while fatigue, thrombocytopenia and hand-foot syndrome had a higher incidence in the sunitinib group (99,104).

Bevacizumab is an antibody that binds to VEGF and has displayed anti-tumour activity in advanced renal cell carcinoma, prolonging the time of disease progression in these patients (105). With the aim to evaluate the combination of bevacizumab with interferon and interferon

monotherapy respectively, 641 patients were selected in a phase III trial, which report clinical improvements in progression free survival with the combination therapy compared with interferon alfa monotherapy (10.2 months versus 5.4 months). Albeit, in the primary endpoint, overall survival, no significant differences were related. Nevertheless, significant side effects such as hypertension, anorexia, fatigue and proteinuria were more documented in the treatment combination group (106,107).

Likewise, high dose of IL-2 can be considered as a therapy option in good risk patients when these agents are not available. Clinical studies reveal that high doses of this cytokine produced significant improvements in tumour regression and response rates in comparison with low doses or interferon therapy. Nevertheless, a toxicity profile has been associated with IL-2 treatment and for this reason, a careful efficacy and safety analysis is require (108,109).

Still analysing first-line treatment, but now in intermediate and poor risk patients, nivolumab, a programmed death 1 immune checkpoint inhibitor, in combination with ipilimumab, an anti-cytotoxic T-lymphocyte associated antigen 4 antibody, is strongly recommended as standard of care in this field (94). A phase I trial evaluated the efficacy and safety of nivolumab in combination with antiangiogenic tyrosine kinase inhibitors or ipilimumab, which the results displayed a large incidence of high-grade adverse effects in the combination with sunitinib and pazopanib, supporting the treatment with nivolumab plus ipilimumab that demonstrated remarkable antitumor activity with durable responses and promising overall survival in patients with metastatic RCC (110,111). Also, a phase III study compared nivolumab plus ipilimumab with sunitinib in untreated advanced clear cell RCC, where patients were treat with nivolumab (3mg/kg) combined with ipilimumab (1mg/kg) intravenously every 3 weeks for four doses, followed by nivolumab every 2 weeks, or sunitinib (50 mg) once daily for 4 weeks (6-week cycle) (112). Outcomes reveal that nivolumab plus ipilimumab achieved higher overall survival, progression free survival and objective response rates, as well as significant improvements in quality of life among intermediate and poor risk patients (113). Nonetheless, the results in patients with good risk displayed superior progression free survival and antitumor responses in sunitinib arm, suggesting that front-line therapy differ as patient profile (92).

In addition, most patients treated with VEGF-targeted drugs have developed resistance and consequently, progression of disease have been reported in clinical practice. Cabozantinib, an oral small-molecule inhibitor of tyrosine kinases, which not only neutralizes VEGF but MET and AXL, two oncoproteins that normally are upregulated in von Hippel-Lindau deficient RCC and associate with poor prognosis, was compared with sunitinib as first-line treatment in patients with intermediate and poor risk features. In this phase II trial, cabozantinib achieve longer progression free survival (8.2 months) than sunitinib (5.6 months) and the superiority in response rates might be a reflexion of cabozantinib-target action. Moreover, the predominant adverse effects reported in both groups comprised diarrhea, hypertension, fatigue, hematologic abnormalities and palmar-plantar erythrodysesthesia. In this way, cabozantinib demonstrated superiority when compared with sunitinib in intermediate and poor risk patients (114).

In poor risk patients, under certain circumstances, temsirolimus, an inhibitor of mammalian target of rapamycin (mTOR) kinase is recommend. This signalling protein regulate cells growth and proliferation. In order to evaluate the efficacy and safety, a phase III trial was performed where temsirolimus was compared with interferon alfa monotherapy or with the combination of both agents. Improvement in overall survival in patients with advanced RCC and poor

prognosis treated with temsirolimus was the principal finding, achieving 10.9 months compared with 7.3 months of interferon and 8.4 months of combination therapy, respectively. This mTOR inhibitor proved to be superior to the combination with interferon alfa and presented less adverse reactions profile among patients with metastatic RCC and poor risk factors (115).

To close the analysis of first-line treatment options, a summary is present on figure 5.

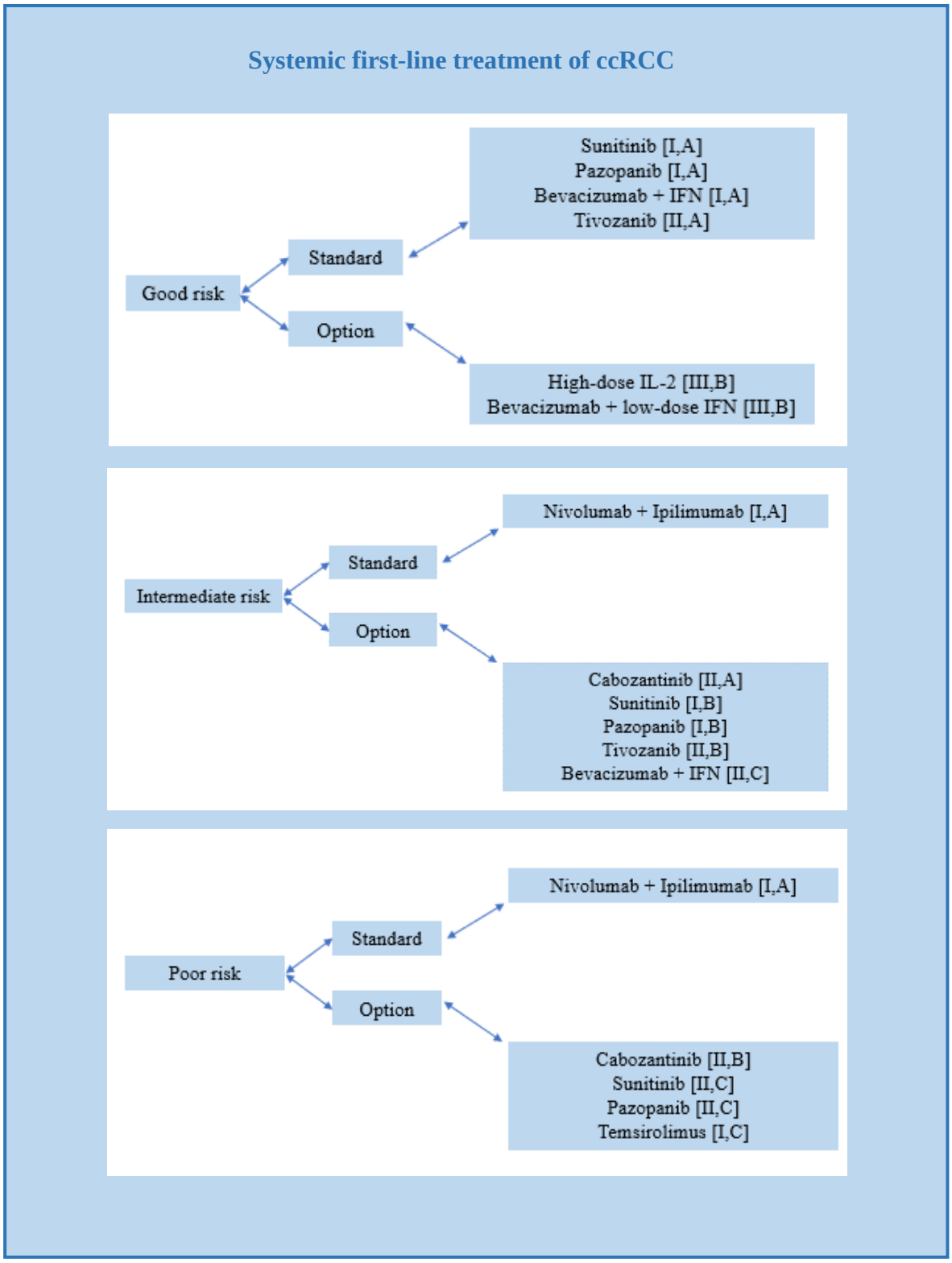

Figure 5 – Systemic first-line treatment of clear cell renal carcinoma for each prognosis classification. Adapted from ESMO guidelines (94).

Focusing attention in second-line treatment it is possible to identify two subgroups. If patients were already treated with tyrosine kinase inhibitors drugs, nivolumab, cabozantinib and tivozanib are the standard of care and axitinib, everolimus and lenvatinib combined with everolimus are the available options when the first ones are not. On the other hand, if patients were treated with nivolumab plus ipilimumab, options such as the combination of lenvatinib with everolimus or any tyrosine kinase inhibitor therapy are suggested (94).

The results of everolimus trials indicate that resistance to VEGF inhibitors does not imply resistance to mTOR inhibitor agents. This drug was studied in patients with advanced RCC with disease progression after VEGF-target therapy (116). Side by side with placebo, everolimus therapy obtained real outcomes in progression free survival (4.9 months versus 1.9 months) in patients that had progressed with prior VEGF-target therapy. The median overall survival was 14.8 months in everolimus arm versus 14.4 months in placebo arm (117). However, notable adverse effects were reported among patients treated with everolimus, such as rash, stomatitis, diarrhea and non-infectious pneumonitis, a serious side effect correlated with rapamycin target treatment (116). In addition, with the aim to study nivolumab and everolimus activities in patients previously treated with antiangiogenic agents, 821 patients with metastatic RCC were randomized to receive nivolumab (3 mg/kg intravenously every 2 weeks) or everolimus (10 mg orally once a day) (118). The results of overall survival (25.0 months versus 19.6 months) and the objective response rates (25% versus 5%) that were documented favoured nivolumab clinical use in these patients (119). The most commonly adverse effects were fatigue in nivolumab group and anemia in everolimus group (118). In this way, outcomes stablished the use of everolimus as an option in second-line setting. In like manner, an interesting studied compare first line everolimus followed by sunitinib with the recommend sequence of first line sunitinib followed by everolimus in patients with advanced RCC. In the primary endpoint, progression free survival was longer with sunitinib-everolimus sequence than everolimus-sunitinib scheme (32.0 months versus 22.4 months), as well as when use as first line, everolimus demonstrated a median progression free survival of 7.9 months, whereas sunitinib achieved 10.7 months, supporting the standard paradigm of everolimus therapy after progression in patients treated with sunitinib (120,121).

Furthermore, in order to compare two option treatments in second line setting, cabozantinib and everolimus were studied in a randomized phase III trial, where 658 patients received cabozantinib at a dose of 60 mg daily or everolimus at a dose of 10 mg daily (122). Cabozantinib improved overall survival (21.4 months versus 16.5 months), progression free survival (7.4 months versus 3.8 months) and objective response rate (21% versus 5%) in patients with advanced RCC who undergone disease progression subsequently to VEGF-target therapy (123). Additionally, the most common adverse effects observed in cabozantinib arm were the same as reported in others clinical trials with VEGF tyrosine kinase inhibitors, while in everolimus arm, pneumonitis, peripheral edema, anemia and hyperglycemia were the predominant documented (122).

Likewise, when compared cabozantinib with the other therapeutic classes agents , this agent proved to be superior, except with nivolumab, the standard of care in patients with disease progression and previously treated with VEGF target therapy (94,124).

As proved above, nivolumab demonstrated advantage over cabozantinib and everolimus in second line context for metastatic RCC in patients previously treated with antiangiogenic drugs. Since immunotherapy specific-patient response and its pharmacological mechanism of action are different from the other therapeutic classes, the therapy with nivolumab beyond progression in patients treat with this drug heretofore was investigated. Data identified tumour reduction post-progression in conjunction with positive features and acceptable safety profile in patients treated with nivolumab already (125).

These last observations mentioned support the use of nivolumab as standard of second line therapy in patients with advanced RCC and in those with notable symptomatic improvements despite progression of disease. Nevertheless, an accurate analysis should be done to determine which are the best patients who will benefit and the optimal duration of therapy.

To complete this topic, a summary of second line treatment is present on figure 6.

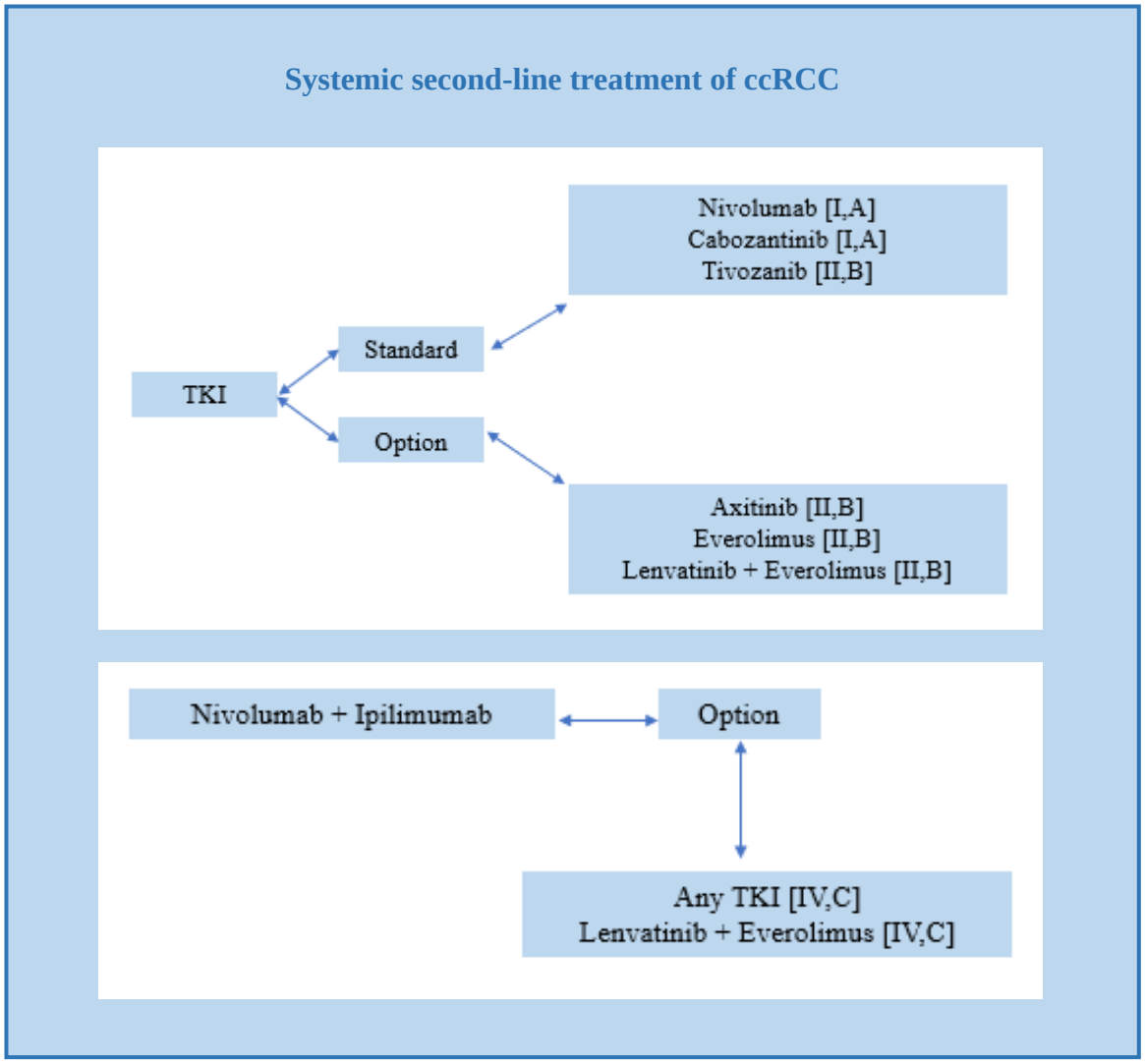

Figure 6 – Systemic second-line treatment of clear cell renal carcinoma based on first-line therapy. Adapted from ESMO guidelines (94).

In a way to end this chapter, it is important to highlight the third line therapy. According to first and second line treatment that patient previously performed, a third option is recommend based on remaining therapeutic classes. Additionally, the patient still has the opportunity to join to current clinical trials (94).

To conclude, a summary of third-line therapy is display on figure 7.

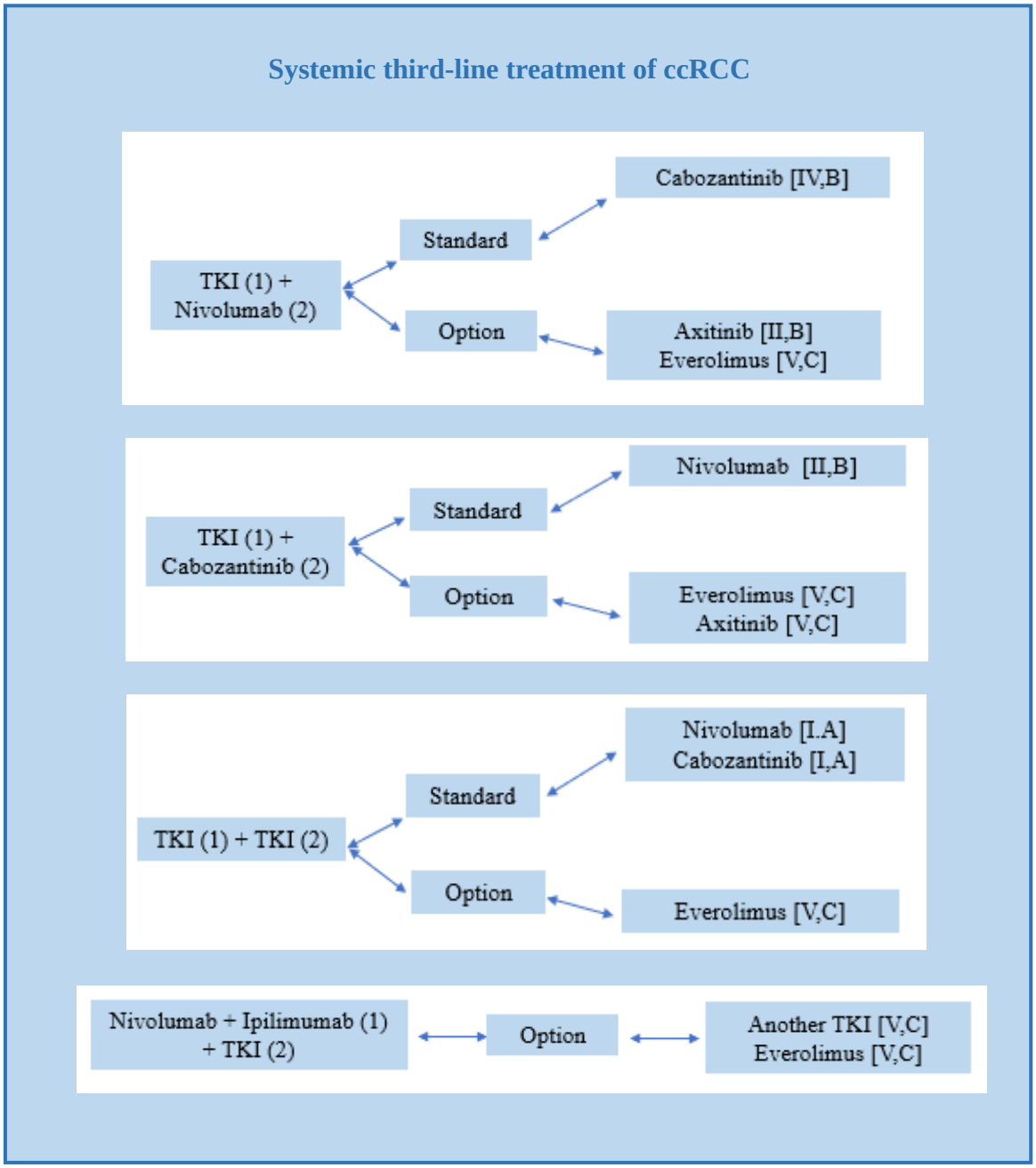

Figure 7 – Systemic third-line treatment of clear cell renal carcinoma based on first line and second line therapy. (1) – first line treatment, (2) – second line treatment. Adapted from ESMO guidelines (94).

As a complementation, a synthesis of the scientific studies cited above and their results are presented in the following table.

Table 4 – Results of pivotal trials of distinct therapeutic classes in metastatic renal cell carcinoma (92,95,104,110,112,114–116,118,122).

Study	Experimental arm	Control arm	Overall survival (months)	Progression free survival (months)
Sutent	Sunitinib	IFN	26.4 vs 21.8	11 vs 5
Pazopanib and placebo	Pazopanib	Placebo	22.9 vs 20.5	9.2 vs 4.2
Comparz	Pazopanib	Sunitinib	28.3 vs 29.1	8.4 vs 9.5
Bevacizumab and IFN	Bevacizumab + IFN	Placebo + IFN	23.3 vs 21.3	10.2 vs 5.4
Checkmate 214	Nivolumab + Ipilimumab	Sunitinib	Not reach vs 26.0	11.6 vs 8.4
Cabosun	Cabozantinib	Sunitinib	26.6 vs 21.2	8.2 vs 5.6
Temsirolimus and IFN	IFN + Temsirolimus/ Temsirolimus	IFN	8.4 vs 10.9 vs 7.3	4.7 vs 5.5 vs 3.1
Everolimus and placebo	Everolimus	Placebo	14.8 vs 14.4	4.9 vs 1.9
Checkmate 025	Nivolumab	Everolimus	25 vs 19.6	4.6 vs 4.4
METEOR	Cabozantinib	Everolimus	21.4 vs 16.5	7.4 vs 3.9

3.9. Systemic therapy for non-clear cell RCC histology

Non-clear cell renal cell carcinomas are characterized as a heterogenous malignancies of kidney cancers that broadly differ on morphology and histology features, genetic profile, clinical development and prognosis. Clinical data are restrained to evidences in patients with clear cell cancers and then studied and tested in these rare histological subtypes. Therefore, the optimal therapy algorithm is still doubtful and the enrolment in clinical trials is strongly recommend is these patients (126,127).

Furthermore, the majority of scientific data available is generally focus on TKI or mTOR inhibitors agents and consider non-clear cell tumours as a single homogeneous population rather than concentrate in each molecular pathway and studied unique approaches for each subtype (126). For these reasons, no detailed treatment recommendations can be graded in this field (94).

The greatest part of patients who have been enrolled in clinical trials presented papillary and chromophobe cancers. Generally it was report that both VEGF receptor inhibitors and mTOR inhibitors based therapies proved efficacy and benefit in these patients (127,128). However, sunitinib achieved better outcomes, improving progression free survival and overall survival, and therefore demonstrating a modest superior in comparison with mammalian target of rapamycin agents (129).

Papillary renal cell carcinomas represent the second commonly type of kidney cancers. Nevertheless, the scientific evidences of drugs efficacy are still scarce and the molecular mechanisms are limited to the involvement of MET mutations in development and progression of this histological subtype (130–132). In addition, papillary malignancies are subdivide into type 1 and 2 respectively, which outcomes are better in type 1 (133). Relevant studies report that either sunitinib or everolimus have demonstrated promising improvements in progression free survival and overall survival, as well as favourable safety profile (133,134).

In the same way, chromophobe renal cell carcinomas responded propitiously to sunitinib treatments. Nevertheless, this histologic subtype is characterize by mutations on chromosome 7 involved in the mTOR pathway, and for these reason, present clinical sensitivity to rapamycin analogues (128). Therefore, everolimus reported notable efficacy in these patients, independently of previous VEGF therapy and in some cases, sequential treatments can lead to positive outcomes (135,136).

Also, in these two subtypes, cabozantinib indicated encouraging activity after disease progression, while medullary and collecting duct malignancies have been reported to be refractory to tyrosine kinase inhibitors drugs (137). Likewise, pazopanib achieved good responses and demonstrated tolerable safety profile in non-clear cell carcinomas trials, with better results in papillary and chromophobe patients (135,138,139).

In opposition, sarcomatoid and collecting duct/medullary carcinomas represent high-grade disease and aggressive clinical development features, which the therapeutic options are limited and only can provide short-term palliation (140–143). In patients with sarcomatoid malignancies, kinase inhibitors have been widely studied and tested with relevant improvements, while forward collecting duct disease presented noteworthy responses with chemotherapy-based therapy, which should be considered as the standard regime (143–145).

Interestingly, cytotoxic treatment is inactive in patients with clear cell and less aggressive non clear cell histologies (144,146). Additionally, the results suggest that the combination of antiangiogenic therapy and cytotoxic chemotherapy is well tolerable and is more efficient, improving survival in patients with metastatic disease (147,148).

Finally, pertinent data support the use of nivolumab for distinct patients with metastatic non-clear cell carcinomas. Nivolumab presented notable anti-tumour activity and evident responses in a heterogeneous population of patients (149).

It is important to note that non-clear cell renal cell carcinomas are an area that require further investigation, centre in each histology group as a single disease with unique molecular pathways and with considerable differences in risk and prognosis profile (126,128).

In conclusion, a summary of first-line therapy of non-clear cell RCC is illustrate on figure 8.

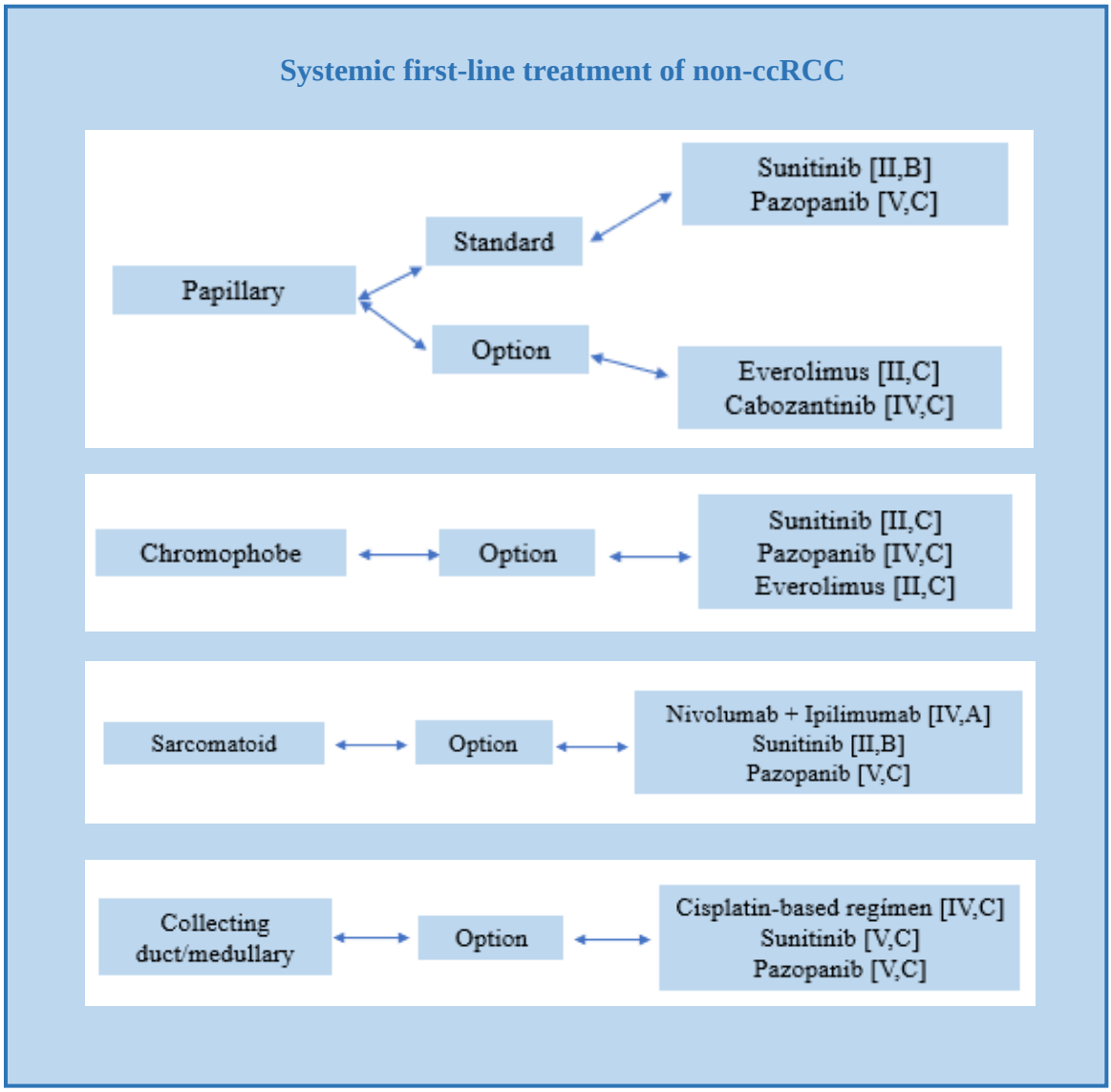

Figure 8 – Systemic first-line treatment of non-clear cell renal carcinoma. Adapted from ESMO guidelines (94).

4. Materials and methods

In cooperation with São Francisco Xavier Hospital it was possible to analyse the use of immunotherapy in four patients with renal cell carcinoma. In this way, several crucial data were collected to perform this analysis, of which we highlight: the age of the patient, the gender, risk factors, the results of staging and prognosis classification models, if patients underwent a nephrectomy procedure, the distinct therapeutic drugs in each line of treatment with the posology, number of cycles and the corresponding duration in months, as well as, the adverse effects reported, if patient`s tumour progress or if culminate into death and if it possible, with the available data, the overall survival estimation.

The strategy of this fieldwork is interpreting the collecting data of the four patients, performing an analysis of the therapeutic decisions, understand if they are in line with the guidelines in force, and compare with applicable and valuable scientific data. Furthermore, specific and recent data were recorded to conclude about the efficacy and safety profile of each drug, with the major focus on benefit/risk ratio of nivolumab, an immunomodulator agent.

As sources of data collection were used medical and pharmaceutical records, which were evaluated the clinical process of each patient, examinations performed and the respective therapeutic approaches. The following diagram presents all the key factors considered indispensable and the reasoning used to proceed with the analyse of the use of the oncologic immunotherapy in a hospital setting.

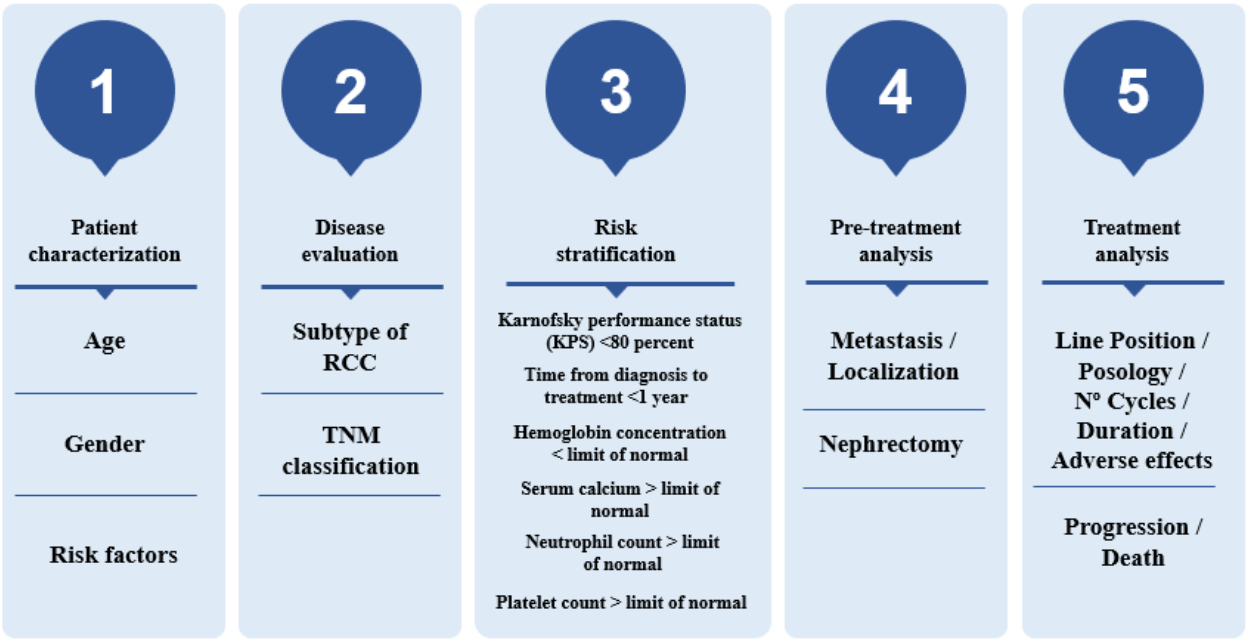

Figure 9 – Summary of the steps and respective information collected during the data gathering process.

In addition, the comparative analysis and respective conclusions were performed using relevant and recent scientific studies, as well as, pertinent clinical trials published in PubMed database, as well as the available guidelines in force in this therapeutic area.

The main conclusions drawn about the efficacy and safety, as well as, the superiority or inferiority between the different medicines authorized to treat patients with renal cell carcinoma diagnosis were accomplished through the outcomes of clinical trials. To understand these same results, it is required to define two central concepts: progression free survival defined as the time from randomization to the first progression or death, and overall survival defined as the time from randomization to death. These two factors were essential to perform the comparative analysis among the different drugs.

Complementary, the data collected from clinical practice were organized and worked on in Microsoft Excel.

In conclusion, a schema of the methodology used in this research work is present on figure 10.

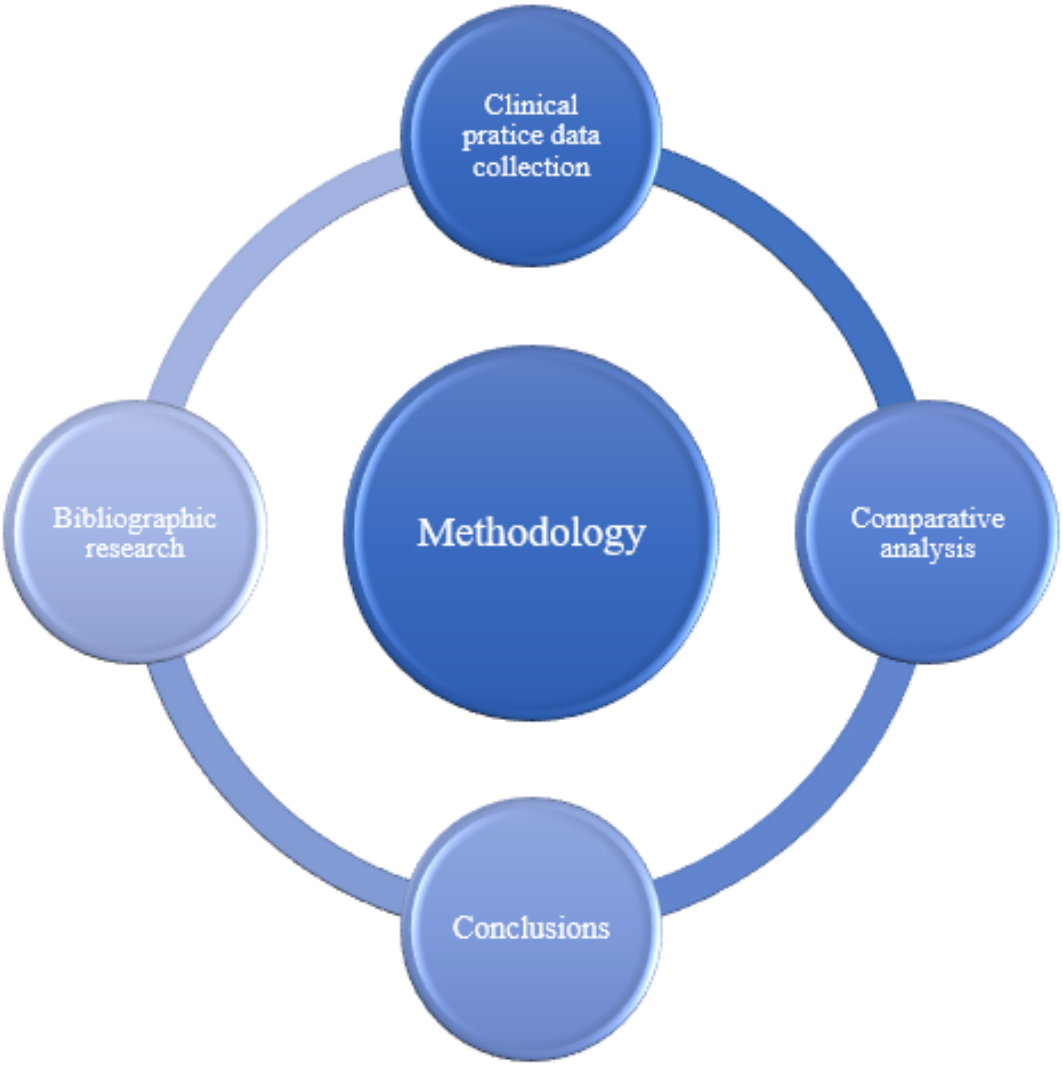

Figure 10 – Synthesis of the work methodology divided into four main phases.

5. Results

The following tables will display the information collected.

Table 5 – Results of patient's characterization phase. The terms cyst and tobacco refer to cystic kidney disease and smoking habits respectively.

Patient	Age	Gender	Risk factors
Patient 1	28	Male	0
Patient 2	39	Male	Cyst
Patient 3	64	Male	Tobacco
Patient 4	56	Male	0

Table 6 – Results of disease evaluation phase.

Patient	Subtype of RCC	TNM classification
Patient 1	Clear cell	IV
Patient 2	Clear cell	IV
Patient 3	Papillary	IV
Patient 4	Clear cell	IV

Table 7 – Results of risk stratification phase.

Patient	Karnofsky performance status (KPS) <80 percent	Time from diagnosis to treatment <1 year	Haemoglobin concentration < limit of normal	Serum calcium > limit of normal
Patient 1	No	Yes	Yes	No
Patient 2	No	No	No	No
Patient 3	No	No	No	No
Patient 4	No	No	No	No

Table 8 – Results of risk stratification phase.

Patient	Neutrophil count > limit of normal	Platelet count > limit of normal	Risk stratification
Patient 1	No	No	Intermediate
Patient 2	Yes	No	Intermediate
Patient 3	No	No	Good
Patient 4	No	No	Good

Table 9 – Results of pre-treatment analysis phase.

Patient	Metastasis / localization	Nephrectomy
Patient 1	Lung	No
Patient 2	Lymph nodes, lung	Yes
Patient 3	Bones, lymph nodes, inter-aorto-cava and left lateral aortic	Yes
Patient 4	Lung, abdominal wall	Yes

Table 10 – Results of treatment analysis phase.

Patient	First line	Posology	Nº cycles	Duration	Progression/ Death	Adverse effects
Patient 1	Pazopanib	400mg 2x/day	4	4 months	Progression	No
Patient 2	Pazopanib	400mg 2x/day	0	14 days	Toxicity	Hepatotoxicity
Patient 3	Sunitinib	50mg D1 - D28, 6/ 6 weeks	18	27 months	Progression	Fatigue, anorexia, hand-foot syndrome, diarrhea
Patient 4	Pazopanib	400mg 2x/day	38	38 months	Progression	No

Table 11 – Results of treatment analysis phase.

Patient	Second line	Posology	Nº cycles	Duration	Progression/ Death	Adverse effects
Patient 1	Axitinib	5mg 2x/day	4	4 months	Progression	No
Patient 2	Sunitinib	50mg D1 - D28, 6/ 6 weeks	4	5.6 months	Progression	Hand-foot syndrome
Patient 3	Nivolumab	159mg 14/14 days	34	15 months	Progression	Cortical-supra renal insufficiency
Patient 4	Axitinib	5mg 2x/day	4	4 months	Progression	No

Table 12 – Results of treatment analysis phase.

Patient	Third line	Posology	Nº cycles	Duration	Progression/ Death	Adverse effects
Patient 1	Nivolumab	267mg 14/14 days	3	1.5 months	Death	No
Patient 2	Nivolumab	225mg 14/14 days	1	0.5 months	Death	No
Patient 3	Axitinib	5mg 2x/day	11	11 months	Death	No
Patient 4	Everolimus	10mg 1x/ day	3	3 months	No response	Mucositis

Table 13 – Results of treatment analysis phase.

Patient	Fourth line	Posology	Nº cycles	Duration	Progression/ Death	Adverse effects
Patient 1						
Patient 2						
Patient 3						
Patient 4	Nivolumab	200mg 14/14 days	13	6.5 months	Progression	No

Table 14 – Results of treatment analysis phase.

Patient	Fifth line	Posology	Nº cycles	Duration	Progression/ Death	Adverse effects
Patient 1						
Patient 2						
Patient 3						
Patient 4	Everolimus	10mg 1x/ day	6	6 months	Progression	No

Table 15 – Results of treatment analysis phase.

Patient	Sixth line	Posology	Nº cycles	Duration	Progression/ Death	Adverse effects
Patient 1						
Patient 2						
Patient 3						
Patient 4	Cabozantinib	10mg 1x/ day	Still doing	Still doing		Diarrhea, fatigue and hypertension

Table 16 – Overall survival estimation with the available information.

Patient	Overall survival
Patient 1	11 months
Patient 2	14 months
Patient 3	
Patient 4	

6. Discussion

Firstly, it should be noted that the population is not representative enough to draw assertive conclusions, however, is heterogeneous enough to perform a very fascinating analysis.

The population in study is constituted by only male elements but with a large range of ages. An interestingly point to highlight here is that kidney cancers are more prevalent in man than in women. Furthermore, two patients present risk factors, which goes according to the scientific studies that report the predisposition to develop renal cell carcinomas in the presence of certain risk factors.

Focusing attention on stage and prognosis classification models, both patients present a stage IV stablished by TNM grades, since in all were recorded metastases at diverse sites. Additionally, the risk stratification was calculated based on International Metastatic RCC Database Consortium formula. Afterwards the prognosis assessment, it was documented that two patients achieved a good risk and the other two an intermediate risk classification respectively.

Advancing with the discussion, this one will be divided according to the subtype of RCC and the risk stratification of each patient. Starting with patient 4, a 56 years old man with no risk factors, diagnosed with stage IV clear-cell RCC and classified with good risk profile. This patient initiated pazopanib as first line treatment at 400mg twice a day and performed 38 cycles during 38 months without any adverse effects. After tumour progression, the patient started the second line of treatment with axitinib 5mg twice a day, completing 4 cycles lasting 4 months. Once again without any adverse effects and in presence of tumour progression, everolimus was introduced as a third line of therapy at 10mg once daily for 3 cycles for 3 months. In addition, mucositis was reported as the principal adverse effect and afterward no response, the patient initiated nivolumab at 200mg every 14 days for 13 cycles corresponding to 6.5 months of treatment. Despite no reported adverse effects, the tumour progressed and for this reason everolimus was reintroduced for 6 months. Following further disease progression, cabozantinib was introduced at 10mg once daily and until the date of data collection still remained this treatment with some notorious side effects such as diarrhea, fatigue and hypertension.

Examining the therapeutic decisions using the information presented in the chapters above and the ESMO guidelines, it possible recognize that the introduction of pazopanib as the first line of treatment is in accordance with them. Moreover, the patient had excellent responses, with ample progression free survival and without any adverse effects. Notwithstanding the fact that axitinib is considered an option in second line setting, as well as, everolimus in third line position after two lines of tyrosine kinase inhibitors therapy, the agents that should have been selected in both lines (second and third) are nivolumab or cabozantinib with evidence of superior efficacy and clinical benefit. It is important to emphasize that everolimus in third line after a first and a second line treatment with TKI agents, had not disclosed in sufficiently large studies relevant efficacy and clinical benefit in this position, and for these reasons can be justified the non-response to everolimus treatment in this patient. With the subsequent introduction of nivolumab, the progression free survival outcomes increased remarkably with no associated adverse effects. These results clearly show the clinical superiority of nivolumab over axitinib and everolimus.

Interestingly, following tumour progression and reintroduction of everolimus subsequently to nivolumab therapy, the patient started to respond to the drug and achieved a progression free survival of 6 months without side effects. Here are pertinent open questions about the possible effect of nivolumab on the effectiveness of other agents in this therapeutic field.

As last line, patient 4 initiated cabozantinib and until the time of data collection the patient was under treatment with significant adverse effects documented, such as diarrhea, fatigue and hypertension. Thus, regarding to the toxicity profile, nivolumab is superior to cabozantinib. Nonetheless, not exist enough data to conclude about the efficacy profile between the two agents. Evidently and proved by this patient, nivolumab demonstrate superiority in RCC patients with clear cell histology and good prognostic features after first line treatment with TKI agents. Likewise, nivolumab proved in this patient a clinical benefit higher than the associated risk.

Concentrate now in patient 1 and 2, both classified with a stage IV clear-cell RCC and intermediate risk features. The first one is 28 years old and the second 39 years old, both men respectively.

Analysing patient 1, pazopanib was selected to first line therapy at 400mg twice a day during 4 cycles for 4 months. Any adverse effects were reported and after progression, the patient started axitinib in second line position at 5mg twice a day during, as well, 4 cycles for 4 months with any side effects. With subsequent disease progression, nivolumab was introduced at 267mg every 14 days for 3 cycles during 1.5 months without any adverse effects documented. Unfortunately, the treatment ended with the patient's death.

Similarly, patient 2 started pazopanib as first line of treatment but only for 14 days due to the hepatotoxicity unleashed by this drug. Thereafter, pazopanib was replaced by sunitinib at 50mg once daily for 5.6 months. Afterwards tumoral progression and hand-foot syndrome exacerbations, nivolumab was introduced at 225mg every 14 days and after 0.5 months and no evidences of side effects, the patients die.

According to the analysis of the bibliographic references cited above (table 4), in both patients all therapeutic classes used displayed poor progression free survival and overall survival results. One possible explanation is that, conforming American and European guidelines (94,150), pazopanib and axitinib are available options with good evidences in intermediate risk setting, however the standard of treatment in these patients is nivolumab in first line position (patient 1) and in second line after a first line with TKI agents (patient 2). Also, the great part of scientific studies that investigate therapeutic approaches in intermediate and poor risk patients with renal cell carcinoma, highlight the superiority of nivolumab in relation to TKI drugs in these prognosis profiles. Therefore, the poor outcomes reported in these patients with these characteristics accentuate the importance of the introduction of nivolumab as first treatment.

Furthermore, in patient 2, the safety profile of nivolumab is noticeable superior in comparison with pazopanib and axitinib profiles, since these agents triggered significant adverse effects. In addition, in patient 1, any toxicity was documented with any therapeutic agent. In this way, in both patients nivolumab proved a large benefit/risk ratio. Importantly to note that, in order to draw more accurate conclusions about the superiority of the efficacy profile of nivolumab, it is required to study patients with intermediate risk features treated with nivolumab as first line therapy.

Notoriously, both patients have identic therapeutic approaches and similar risk stratification profile but differences in overall survival were remarked, since patient 1 achieve 11 months and patient 2, 14 months respectively. Complementary, it was reported that patient 2 performed a nephrectomy procedure before treatment in contrast to patient 1. This is an interesting topic since some studies reveal that nephrectomy procedures extend overall survival time (151).

Finally, the patient 3, a 64 years old man diagnosed with a stage IV non-clear cell RCC with a papillary histology and classified as a good risk patient, which the main risk factor identified was the smoking habit. Therefore, the patient started sunitinib at 50mg once daily for 4 weeks (6-week cycle) for 27 months and after tumoral progression and in presence of side effects, nivolumab was introduced at 159mg every 14 days during 34 cycles for 15 months. In the course of nivolumab therapy, a cortical-supra renal insufficiency was reported and after further disease progression, axitinib was selected at 5mg twice a day during 11 months with any adverse effects documented. By this time the patient`s death was reported.

In agreement with the guidelines in force, sunitinib as first line of treatment is the best choice as the remaining options have scarce evidence of efficacy and safety when compared with this drug. The prove is that this patient with this subtype of RCC, achieved an outstanding progression free survival time. Nevertheless, the toxicity profile was really significant with fatigue, anorexia, hand-foot syndrome, and diarrhea as the main adverse effects.

Before proceeding with the analysis, it must be underlined that the European and American guidelines are organized differently in patients with distinct histologies than clear cell. The European guidelines list the recommendations by histological subtype and the American guidelines cluster all non-clear cell histologies into one group only. This is important because European guidelines do not refer to nivolumab as an option in patients with papillary histology. Howbeit, American guidelines consider it as one of the main options in patients with non-clear cell histologies. Complementary, the available data confirm that nivolumab has noteworthy antitumoral activity in these patients. In fact, patient 3 present a remarkable progression free survival time, which support the benefit of nivolumab in patients with papillary renal cell malignancies. Nonetheless, a severe adverse effect was reported (cortical-supra renal insufficiency), and so this raises the question whether the benefit of nivolumab in these patients outweighs the risk associated.

Likewise, axitinib is only indicated as an option in this setting in the American guidelines. However, patient 3 had a good response to the treatment with a progression free survival above expectations with any adverse effects described. While on the subject, of the three therapeutic approaches, axitinib was the only that not disclosed toxicity for the patient. Notwithstanding, nivolumab was the medicine that achieved the better efficacy outcomes in this patient, but in terms of the safety profile there are some doubts whether in patients with papillary subtype of RCC, the benefit of nivolumab therapy outweighs the risk associated.

In conclusion, as a global analysis, is important to emphasize that in three of the four patients studied, nivolumab demonstrated a clinical benefit higher than the risk associate and, in the one where doubts remained, the activity of this medicine require further investigation and clinical tests. In addition, concern to the safety profile, nivolumab only presented adverse effects in one of the four patients. It is possible to deduce that the benefit/risk profile of nivolumab is remarkable.

7. Conclusions and future perspectives

Annually, over than 330.000 patients are diagnosed with renal cell carcinoma and approximately 140.000 deaths are reported (122). Therefore, new therapeutic approaches in this area with so much to investigate are imperative.

Oncologic immunotherapy is an innovative and promising therapeutic approach that stimulate the natural immune system ability to fight cancer. The central goal is restoring, preserve and improve the immune cycle through strategies such as, enhancement of antigen presentation, cells proliferation and differentiation, as well as, blockage of tumoral suppressive processes.

Distinct are the immunotherapy approaches in development, however one of the most promising is immune checkpoints inhibitors. Nivolumab is one of the principal drugs, which belongs to this therapeutic class, and recommended as the standard of treatment in patients with renal cell carcinomas. This human immunoglobulin monoclonal antibody has proved outstanding clinical outcomes.

Despite the population studied in this fieldwork was small, nivolumab demonstrated in most of the elements, encouraging and significant clinical benefits that outweighs the risk associated. In this same heterogeneous sample, nivolumab proved positive efficacy and safety profile with suitable benefit/risk ratio. Nevertheless, to draw more assertive conclusions, a continuous work must be performed in future.

In this way, further analysis should be accomplished in the future to support this data, namely the efficacy of nivolumab in patients with intermediate or poor risk profiles, treated with this drug in first-line setting. In addition, some questions remain to be answered, particularly in patients with non-clear cell histologies, where the function of nivolumab in each unique subtype is not completely studied and evaluated.

One of the biggest challenges forward will be to understand the role of cancer immunotherapy in each histological subtype of kidney cancer, considering them as single diseases, and apply this knowledge to different patient profiles. Likewise, the identification of new and valid biomarkers is required to support clinical decisions and predict the best responses.

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